Systematic Exploration of the Antigen Binding Activity of Synthetic Peptides Isolated from the Variable Regions of Immunoglobulins*  

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Sets of short (12 residues) cellulose-bound synthetic overlapping peptides derived from the sequences of the variable regions of the heavy and light chains of three different antibodies (an anti-thyroglobulin antibody, the HyHEL-5 anti-lysozyme antibody, and an anti-angiotensin II antibody) were used to systematically assess the antigen binding capacity of peptides from the antibody paratope outside their natural molecular context. Peptides enclosing one or several of the complementarity determining region (CDR) residues had antigen binding activity, although the most active peptides were not necessarily those bearing the greatest number of CDR residues. Several residues from the framework region, preceding or following the CDR, were found to play a role in binding. Affinity constants from 4.1 × 10⁻⁷ to 6.7 × 10⁻⁹ M⁻¹ for the soluble form of 9 lysozyme-binding dodecapeptides were measured by BIACore analysis. Alanine scanning of lysozyme-binding hexapeptides from the HyHEL-5 sequence identified 98 residues important for binding, of which 22 corresponded to residues that had been shown by x-ray crystallography to be at the interface between HyHEL-5 and lysozyme. Our results could be of interest for the rational identification of biologically active peptides derived from antibody sequences and in providing an experimental basis for mutagenesis of the antibody paratope.

Antibody molecules bind antigens with high affinity and specificity by synergistically using multiple noncovalent forces. The combining site (paratope), whose shape is complementary to the epitope on the antigen, is made up of the hypervariable regions, also called complementarity determining regions (CDRs) (1). It is commonly accepted that there are three CDRs in the light chain (L1, L2, and L3) and in the heavy chain (H1, H2, and H3). These CDRs fold into turn structures that are stabilized by the β-sheet framework of the variable domains. The interface between antibodies and antigens has been precisely described by x-ray crystallographic studies, and several complexes between Fab fragments of monoclonal antibodies and peptide or protein antigens have been recently described (for reviews see Refs. 2–4). The structures of antibody-antigen complexes indicate that at least four of the CDRs, and in some cases all six CDRs, contribute to antigen binding (5). Residues in the framework have rarely been reported to participate in this interaction (6, 7).

Antibody-peptide or antibody-protein complexes are excellent model systems to study the physicochemical requirements for molecular recognition. Unfortunately, it is a difficult task to obtain crystals suitable for the structural elucidation of antibody fragments in complex with proteins or peptides. Therefore, other approaches to obtain information about the key residues involved in the interaction would be very useful, in particular for paratope mutagenesis. Some workers have demonstrated that synthetic peptides derived from the amino acid sequences of CDRs bind antigens with specificities similar to those of the original antibody molecules (8–15). Such peptides have very often been chosen in the CDR3 of the VH sequence, which is considered to play a prominent role in defining antibody reactivity. However, the capacity of synthetic peptides derived from the variable regions of a given antibody to bind an antigen has never been probed in a systematic manner, i.e. in assessing the antigen binding capacity of every overlapping peptide from the VH and VL sequences.

In this study, we present the results obtained by measuring the ability of an antibody to bind to sets of immobilized overlapping peptides of uniform size covering the amino acid sequences of the VH and VL domains of three different antibodies. The peptides were prepared by the Spot method (16), which has previously been successfully used to identify peptide epitopes recognized by anti-protein antibodies and further developed to map protein-protein interaction sites (17). Our results indicate that numerous peptides show antigen binding capacity, most of them exhibiting measurable affinities in BIACore, and that paratope residues important for antigen recognition can be identified by peptide analysis.

EXPERIMENTAL PROCEDURES

Antigens—Hen egg white lysozyme was from Sigma, and human thyroglobulin was from UCB-Pharma (Nanterre, France). Synthetic angiotensin II and an N-terminally biotinylated derivative were prepared as described below.

Protein Biotinylation—2 mg of the antigen in 2 ml of bicarbonate buffer (pH 8.6) were biotinylated by using a commercial reagent (Amer sham RPN2202) according to the manufacturer’s protocol. Biotinylated antigens were incubated with 0.1 M glycine (1 h, 37 °C) and then stored in phosphate-buffered saline at −20 °C.

Amino Acid Sequences of Antibodies—The numbering of the amino acid sequences of the variable regions was that of Wu and Kabat (1). The amino acid sequence of the anti-angiotensin II antibody 4D8 was established by sequencing the cDNAs corresponding to the heavy and light chains after reverse transcription of the mRNA from the 4D8 hybridoma (18). The amino acid sequences of the anti-lysozyme anti-
body HyHEL-5 and the anti-human thyroglobulin Tg10 antibody were taken from the literature (19, 20).

Peptide Synthesis on Cellulose Membrane—The general protocol has been described previously (21). Membranes were obtained from Abimed (Langenfeld, Germany). Fmoc amino acids and N-hydroxybenzotriazole were from Novabiochem. An ASP222 robot (Abimed) was used for the coupling steps. All peptides were acetylated at their N terminus. After the peptide sequences had been assembled, the side-chain protecting groups were removed by trifluoroacetic acid treatment (16).

Immunoassay with Cellulose-bound Peptides—The general protocol was the same as for epitope analysis (16) except for the use of a biotinylated antigen (90-min incubation at 37 °C). After washing the membrane, a 1:3000 dilution of an alkaline phosphatase-streptavidin conjugate (Sigma) was incubated for 30 min at room temperature. Binding was revealed by addition of a phosphatase substrate (5-bromo-4-chloro-3-indolyl phosphate-3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma), giving a blue precipitate on those spots having bound the alkaline phosphatase-streptavidin conjugate.

A plot of spot intensities was obtained with the NIH Image software after scanning the membrane as described (21). Color intensities were calculated with reference to a black spot taken as the maximum of a 0–255 scale (arbitrary units). To allow the reuse of the membrane, it was sequentially treated by dimethylformamide, 6 M urea, and 10% acetic acid in ethanol so as to remove the precipitated dye and molecules bound to peptides. The reactivity of each antigen was assessed in two or three independent experiments.

Synthesis of Soluble Peptides—All soluble peptides were synthesized on an Abimed AMS 422 synthesizer by Fmoc chemistry. Except for angiotensin II, a spacer sequence (YKK) was added at the N terminus of every peptide followed by a biotin residue. Peptides were deprotected and released from the resin by trifluoroacetic acid treatment in the presence of appropriate scavengers. Peptides were lyophilized, and their purity was assessed by HPLC. When necessary, peptides were purified to greater than 90% HPLC homogeneity.

Real Time Analysis of Peptide-Antigen Interaction by BIAcore—The BIAcore apparatus was from BIAcore (Uppsala, Sweden). All experiments were carried out at 25 °C. N-terminally biotinylated peptides (5 pmol/mg) in Hepes-buffered saline buffer were immobilized on a streptavidin-coated sensor chip. The injection was performed at a flow rate of 5 μl/min. The net surface plasmon resonance signal for immobilized peptides was found to be about 25–30 resonance units after completion of the chip regeneration cycle, which corresponds to 25–30 pg/mm² (12–15 fmol/mm²). The binding kinetics of lysozyme to immobilized peptides was determined by injecting lysozyme (2–4 pmol) in Hepes-buffered saline buffer (running buffer) at a flow rate of 10 μl/min. Dissociation was observed in running buffer without dissociating agents at a flow rate of 10 μl/min. The kinetic parameters of the binding reactions were determined using BIAsolution software (22). The dissociation rate (off-rate) constant k₂ was determined from a plot of ln[abs(DR)/R] versus time, R being the surface plasmon resonance signal at time t; the association rate constant (on-rate) k₁ was determined from a plot of ln(abs(dR/dt)) versus time. The apparent equilibrium dissociation constant was calculated from the kinetic constants: K_D = k₂/k₁.

Analysis of Framework-CDR Interaction by Molecular Modeling—The coordinates of the HyHEL-5-lysozyme complex (3hf; Ref. 23) were used with the Insight II software to identify CDR residues in contact (i.e. less than 3.4 Å apart) with amino acids from the framework that were found to be important by Spot peptide analysis.

RESULTS

Capacity of Peptides Derived from the V_H and V_L Sequences of Three Different Antibodies to Specifically Bind the Cognate Antigen—The capacity of short peptides derived from the variable regions of three different antibodies to bind the cognate antigen was investigated in a systematic manner. The V_H and V_L amino acid sequences were presented as sets of 110 overlapping dodecapeptides (2-residue frameshift) synthesized according to the Spot method (16, 21). In this method, the peptides remain attached to the cellulose membrane used for their synthesis, and their immunoreactivity is probed by incubating the membrane with a solution containing the ligand. As an example, Fig. 1 shows the results obtained with peptides derived from the variable regions of HyHEL-5, an anti-lysozyme antibody (6). Biotinylated lysozyme (1 μg/ml; 6 × 10⁻⁸ M) bound to several peptides derived from the V_H and V_L sequences of HyHEL-5 (Fig. 1A). A detailed analysis of this interaction is provided under "Relationships between the Sequence of Antigen-binding Peptides and CDR Location in the HyHEL-5 Model." The binding pattern was not affected by incubating the biotinylated lysozyme in a 50-fold dilution of normal human serum, i.e. in the presence of a high concentration of proteins unrelated to lysozyme (Fig. 1B). However, when lysozyme (100 μg/ml) was added to the incubation mixture, binding no longer occurred (Fig. 1C). No binding was observed with the alkaline phosphatase-streptavidin complex (Fig. 1D) except on two control peptides that have the HPGQ sequence recognized by streptavidin (24). The binding of lysozyme to immobilized peptides is therefore specific.

In the second model studied, biotinylated thyroglobulin (1 μg/ml; 3 × 10⁻⁹ M) was also found to bind several peptides from the V_H and V_L domains of the anti-thyroglobulin antibody Tg10 (25). The reactivity was observed in six regions of the membrane that broadly corresponded to peptides containing CDR residues (data not shown). The reactivity was abolished in the presence of an excess of thyroglobulin.

In another set of experiments, the V_H and V_L domains of the high affinity anti-angiotensin II antibody 4D8 (K_D = 1.3 × 10⁻¹¹ M; 18) were scanned by overlapping dodecapeptides. Biotinylated angiotensin II (1 μg/ml; 1 × 10⁻⁶ M) bound to peptides corresponding broadly to the three CDRs of V_L and to H1, whereas peptides corresponding to H2 and H3 showed less...
intense reactivity (not shown). When biotinylated angiotensin II (1 \mu g/ml) was incubated with 1 mg/ml angiotensin II, the signal markedly decreased. No cross-reactivity was observed with any of the three antigens on peptides derived from unrelated antibodies. All of these results showed that 12-mer peptides derived from the amino acid sequence of the \( V_H \) and \( V_L \) domains of three different antibodies are capable of being specifically recognized by their cognate antigen.

Relationships between the Sequence of Antigen-binding Peptides and CDR Location in the HyHEL-5 Model—Table I shows the results of the quantitative analysis of the binding of biotinylated lysozyme to cellulose-bound peptides derived from the amino acid sequence of HyHEL-5. The majority of peptides containing only framework residues displayed no binding activity (peptides 3–8, 20–21, 34–39, 51–58, 84, and 86–87). However, several peptides (31, 33, 34, 40, 59, 68–69, 85, 88–90, and 92–93) that also contained only framework residues were reactive. The possible molecular basis for this reactivity is discussed under “Precise Identification of Residues Contributing to Antigen Binding and Comparison with Contact Residues Defined by X-ray Crystallography.” Analysis of the relationships between the amino acid sequence and the binding properties (Table I) indicated that strong binding capacity was detectable when certain CDR residues were present in the peptide sequence. For example, the sequence VTMTCSSASSSVN from L1 (peptide 12) had no activity, but the following (overlapping) sequence MTCSASSSVNYM (peptide 13) possessed activity; therefore, the motif YM clearly contributed to binding. Other residues from CDRs were thus identified as contributors: DT from L2 at peptide 22, SD from H1 at peptide 60, and YH from H2 at peptide 75. Some residues not belonging to CDRs also apparently contributed to binding activity: GS at peptide 29, YY at peptide 40, TF at peptide 59, KQ at peptide 64, GL at peptide 67, KT at peptide 88, and YY at peptide 92. Also, a decrease in the binding capacity of certain peptides occurred when residues from the CDRs disappeared from the peptide sequence, e.g., the difference in the activities of peptides 19 and 20 could be attributed to the absence of YM in peptide 20. The following CDR residues were thus identified: QW from L3 (difference in binding to peptide 47 versus 48), SD from H1 (peptide 65 versus 66), GS from H2 (peptide 77 versus 78), and DF from H3 (peptide 101 versus 102). When certain residues not belonging to the CDR were removed from the amino acid sequence of a binding peptide, the antigen binding capacity was reduced: absence of RW in the sequence of peptide 26, of FS in peptide 34, of AS in peptide 63, of EW in peptide 74, and of YM in peptide 91. Therefore, it seems that the binding of antigen to cellulose-bound peptides is based on the presence in their sequence of certain residues from the CDRs and in several instances of certain framework residues neighboring the CDRs.

Precise Identification of Residues Contributing to Antigen Binding and Comparison with Contact Residues Defined by X-ray Crystallography—Alanine scanning of hexapeptides derived from each previously identified binding sequence from
the V<sub>H</sub> and V<sub>L</sub> domains of HyHEL-5 was performed to identify the exact residues contributing to antigen binding. The study of the CDR L1 region is given as a detailed example (Fig. 2; see legend of Fig. 3 for the amino acid numbering of the V<sub>H</sub> and V<sub>L</sub> sequences). Three hexapeptides (SSVNYM, SVNYMY, and VNYMYW) and each of their six alanine analogs were synthesized by the Spot method and tested for reactivity with biotinylated lysozyme. Replacing Ser<sup>27</sup> of peptide SSVNYM by an alanine residue led to a failure in the antigen binding capacity, whereas alanine replacement of Tyr<sup>32</sup> led to a disparity in the binding. Changing any of the other amino acids of the peptide SSVNYM did not modify the binding (Fig. 2A). For peptide SVNYMY, the three amino acids Asn<sup>31</sup>, Tyr<sup>32</sup>, and Tyr<sup>34</sup> appeared to be important (Fig. 2B). Alanine scanning of peptide VNYMYW indicated that only Asn<sup>31</sup> is important (Fig. 2C). In preliminary experiments, we noted that the contribution of a given amino acid to binding was not always the same when the surrounding sequence varied, probably reflecting conformational effects. Indeed, Tyr<sup>32</sup> appeared to be a contributor in peptides SSVNYM and SVNYMY but not a contributor in peptide VNYMYW; also, the influence of Asn<sup>31</sup> was apparently more critical in sequence VNYMYW than in other sequences. In fact, several hexapeptides had to be analyzed to define the residues contributing to the binding.

The contributing motif for the L1 region was therefore determined to be 27S--NY-Y<sup>34</sup> (Fig. 3), with all four residues belonging to the CDR. Using the same alanine-scanning approach, contributors were determined for each CDR region. For the L2 region, the motif was identified as 46RWIYD<sup>50</sup> (Fig. 3); one residue (Asp<sup>50</sup>) belonged to the CDR, and four residues were from the framework sequence preceding L2. For the L3 region, the contributing motif was 91WGR-P-F<sup>98</sup> (Fig. 3). Residues Trp, Gly, Arg, and Pro were part of the CDR, Phe<sup>98</sup> being in the framework. For the H1 region, the contributing motif 27Y-FSDYW-EW<sup>36</sup> comprised four residues (DYW-E) from the CDR and four residues from the framework. For the H2 region, the motif comprised a framework residue, Trp<sup>47</sup>, and six residues in the middle of the CDR (<sup>64</sup>S-S-NY<sup>68</sup>HE<sup>61</sup>). For the H3 region, the motif 91YC--HGNYDF-W<sup>103</sup> had five amino acids from the CDR (GNYDF) and four residues from the framework (YC--H and Trp, respectively on the N- and C-terminal sides of the CDR). The determination of critical residues for binding the antigen provided a possible explanation for the reactivity of certain framework sequences; for example, peptides 29–34 (Table I) contain an SGS sequence that is part of the CDR H2; peptides 68 and 69, could be reactive because of the presence of the LEW sequence very similar to the IEW motif of H1. The reactivity of peptide 88 could be due to a strong sequence similarity with peptides 16–18 from the L1 region (SSTAYM as compared with SSVNYM). Thus, a cross-reactivity phenomenon may explain some of the reactivities in framework peptides; however, no similar explanation for the reactivity of peptides 40, 59, 89–90, and 92–93 was found.

The residues identified here as important for the binding of lysozyme to peptides derived from the sequence of the V<sub>H</sub> and V<sub>L</sub> domains of HyHEL-5 were then compared with the residues involved in antigen binding in the crystal structure of HyHEL-5 Fab-lysozyme (6, 23) (Fig. 3). For the L1 region, three amino acids (NY-Y) identified by peptide analysis are implicated in the crystallographic antigen-antibody interaction; Ser<sup>27</sup> was found to be a contributor in our analysis only. For the L2 region, only one CDR amino acid (Asp<sup>50</sup>) was implicated by crystallography in the antigen-antibody complex. Alanine scanning of reactive peptides from this region pointed out the role of both Asp<sup>50</sup> and of the stretch of four residues preceding it. For the L3 region, the 91WGR-P<sup>98</sup> residues of the antigen-antibody complex were identified by peptide analysis with, however, the implication of an additional residue (Phe<sup>98</sup>) For the H1 region, four CDR residues (DYW-E) and one framework residue (Ser<sup>20</sup>) were found by crystallography to be implicated in antigen recognition; by alanine scanning of hexapeptides, the same five amino acids, <sup>30</sup>SDYW-E<sup>36</sup>, were found to be contributors. However, three other amino acids (Y-F-----W) outside the CDR seemed to be implicated in the interaction of the peptide with the antigen. For the H2 region, which is a 17-residue long CDR, seven amino acids from the CDR and one in the framework play a role in the antigen-antibody interaction as determined by x-ray crystallography. By using the Spot method, the importance of three amino acids of the CDR (Ser<sup>54</sup>, Ser<sup>56</sup>, and Asn<sup>58</sup>) and of the Trp<sup>47</sup> residue of the framework was determined. Whereas the crystallographic results implicate the N-terminal part and the middle of the CDR, the C-terminal part of the CDR (motif YHE) seemed to play an important role when peptides
were used. For the H3 region, the motif GNYD found by crystallography is similar to the motif found by the alanine scanning of hexapeptides. However, five amino acids, which were defined by spot peptide analysis as contributors, were not implicated in the HyHEL-5-lysozyme interaction as defined in the crystal structure. Of these five contributors, four of them belonged to the framework (91YC--H95, N-terminal to the CDR, and Trp103, C-terminal to the CDR).

The comparison between residues (totaling 38) important for lysozyme binding to VH- and VL-derived peptides and residues involved in the paratope-epitope interface in the HyHEL-5 lysozyme complex (23). CDR residues are colored in red. Residues labeled with asterisks correspond to residues in direct contact with lysozyme in the crystal structure. A residue was considered to be a contributor if the binding signal was reduced by at least 20% when it was replaced by an alanine. Ab, antibody. The complete amino acid sequences of the VH and VL of HyHEL-5 are given below. CDR residues are underlined.

VL
1  5  10  15  20  25  29  35  40  45  50  55  60  70  75  80  85  90  94  100  105
DIVLTQSPAIMSASPGEKVTMCSAASSSVYMYWQQRKSTPKNWDTKLASSGVPRFSGGSGTSGSTLSLTIMETADAAYGGWWKPNTPFQGGTKLEIK

VH
1  5  10  15  20  25  30  35  40  45  50  52  55  60  65  70  75  80  82  abc  85  90  95  99  105  110
ZVQLQGGSAEMKPGASVKISCKASGTYFSDWVIEWKVQRPQHGLEWIGELI LPGSSGTYHERFKQKATFDATSSTAYMLNSLTEDSGVYYCLH-

![Fig. 3. Comparison of residues found to contribute to the binding of lysozyme to peptides with residues involved in the paratope-epitope interface in the HyHEL-5 lysozyme complex (23).](image)

**TABLE II**

| Contributing residue from the framework | Belonging to the Vernier zone* | Contact residue from the adjacent CDR (CDR#) |
|----------------------------------------|-------------------------------|--------------------------------------------|
| Arg46L                                 | Yes                           | Ala55L (CDR L2)                           |
| Trp47L                                 | Yes                           | Leu44I, Ala55L (CDR L3)                    |
| His61L                                 | Yes                           | Thr211L, Ser221, Lys55L (CDR L2)           |
| Tyr27H                                 | Yes                           | Phe26H, Lys221, Leu55L, Ala55L (CDR L2)   |
| Phe98L                                 | Yes                           | Tyr27H (CDR H1)                           |
| Tyr103H                                | Yes                           | Phe26H, Tyr32H (CDR H1)                    |
| Trp29H                                 | Yes                           | Ser110L, Lys221 (CDR H1)                   |
| His30H                                 | Yes                           | Asp211L (CDR H1)                          |
| His30H                                 | Yes                           | Glu211L, His55L, Pro211L (CDR H3)          |

* Ref. 26.

**Antigen recognition.**

**Affinity Determination of the Interaction between Peptides and Lysozyme by BLACore Analysis**—Based on the sequences of the peptides used in the Spot assay, a series of peptides derived from the variable regions of HyHEL-5 was synthesized by conventional solid phase synthesis and used in BLACore real-time interaction analysis (27). Fig. 4A shows a typical sensorgram of the interaction of an immobilized biotinylated peptide (Lyso 1) with lysozyme; the fitting of the experimental associ-
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Fig. 4. Surface plasmon resonance analysis of interaction between lysozyme and peptide Lyso 1. A, sensorgram for the binding of lysozyme to peptide Lyso 1. B, on-rate constant determination using a one-site model with data from A; the experimental and theoretical curves and the residuals between these two curves are overlaid. C, off-rate constant determination using a one-site model with data from A; the experimental and theoretical curves and the residuals between these two curves are overlaid. RU, resonance units.

Antigen binding occurs through molecular contacts with several of the spatially juxtaposed CDRs of the VH and VL domains of the antibody molecule (28, 29). A systematic evaluation of the capacity of every peptide from the VH and VL regions to bind the antigen was performed in this study to assess the effect of keeping the sequence information but disrupting the precise molecular arrangement of the paratope. It was found that in the case of three different antigens (angiotensin II, hen egg white lysozyme, and human thyroglobulin), differing considerably in their size, numerous immobilized peptides from the VH and VL regions of the cognate antibody bind the antigen in a specific manner. Moreover, we have unpublished results showing that in the case of two other anti-protein antibodies the same observations could be made, arguing in favor of the generality of the phenomenon for anti-protein antibodies. Our present analysis indicates that peptides with antigen binding activity have one or several residues from the CDR in their amino acid sequence. Synthetic peptides enclosing complete CDRs generally displayed strong binding activity; however, peptides representing incomplete CDRs but including amino acids from sequences flanking the CDRs were also active, indicating contribution to the binding of some residues outside the CDR itself. It is not clear whether these residues contribute by directly contacting the antigen or by giving the reactive conformation to the peptide. We observed, however, that these residues often belong to the subset of framework residues that could modulate the conformation of the adjacent CDR (19, 26). It is therefore possible that the conformational state of reactive peptides immobilized on the cellulose membrane is very close to the conformation of the same sequence in the paratope. If this is the case, modification of a peptide residue critical for conformation would affect antigen binding in the same way that mutation of the corresponding framework residue would affect binding by altering CDR conformation. It was observed that some peptides including only framework residues do specifically bind to the antigen; this is possibly due in certain cases to occurrence in the framework sequence of similarities with the motifs contributing to antigen binding. It has been shown that a sequence homology of three residues in a peptide is sufficient to give rise to antigenic cross-reactivity (30). However, in several instances there is no obvious homology so that the chemical or structural basis for this reactivity remains to be assessed.

Binding of nine of thirteen synthetic peptides corresponding to those active in the Spot assay was observed by BIAcore real time interaction analysis. The dissociation constants of the interaction between soluble peptides and lysozyme in the 70–400 nM range are only 2 or 3 orders of magnitude higher than the $K_D$ of the reaction between the whole antibody and ly-
sozyme (31). The kinetics of binding were characterized by rapid dissociation rates, probably due to a small number of interactions between the peptide and the antigen. To improve the binding capacity of such peptides derived from antibody sequences, it is suggested that cyclization could be useful in locking an active conformer (14, 32). Four peptides that were active in the Spot assay did not give measurable affinities in the BLAcore analysis, indicating that detection of antigen binding could be facilitated by the high density of peptides at the surface of the paper (about 3–11 nmol/mm²; Ref. 16), which is not the case in the BLAcore format where the peptide density is as low as 10 fmol/mm².

The identification of short amino acid sequences recognized by peptide/protein antigens may have important applications for the screening of bioactive peptides based upon antibody sequences (11) in the current trend to redefine the minimal antigen-binding fragment (33) with the intention of preparing miniantibodies (34). Our systematic approach provides a way to select, on an experimental basis, peptide sequences with antigen binding activity. The results indicate that such sequences do not always correspond to peptides containing only CDR residues; in fact, residues from the framework were here found to play a critical role in the activity of CDR-based peptides, extending previous observations (13). Several residues that were found to contribute to peptide-lysozyme interaction were not residues located at the paratope-epitope interface (6, 23), indicating that the binding that was observed with short peptides was consistent with the binding of the antigen to the whole antibody. The capacity of the method we used here to pinpoint residues potentially involved in the antigen-antibody interface could be valuable for the design of experiments aimed at mutating antibody-binding sites; in this context, not knowing exactly whether residues identified as important play a structural or functional role would not be detrimental. We suggest that peptide analysis conjugated with powerful binding site topography prediction methods (35, 36) could be a valuable strategy for antibody engineering.

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