Computational Identification of Antigen-Binding Antibody Fragments

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Computational Identification of Antigen-Binding Antibody Fragments

Anat Burkovitz,* Olga Leiderman,* Inbal Sela-Culang,† Gerardo Byk,‡ and Yanay Ofran*†

Determining which parts of the Ab are essential for Ag recognition and binding is crucial for understanding B cell–mediated immunity. Identification of fragments of Abs that maintain specificity to the Ag will also allow for the development of improved Ab-based therapy and diagnostics. In this article, we show that structural analysis of Ab–Ag complexes reveals which fragments of the Ab may bind the Ag on their own. In particular, it is possible to predict whether a given CDR is likely to bind the Ag as a peptide by analyzing the energetic contribution of each CDR to Ag binding and by assessing to what extent the interaction between that CDR and the Ag depends on other CDRs. To demonstrate this, we analyzed five Ab–Ag complexes and predicted for each of them which of the CDRs may bind the Ag on its own as a peptide. We then show that these predictions are in agreement with our experimental analysis and with previously published experimental results. These findings promote our understanding of the modular nature of Ab–Ag interactions and lay the foundation for the rational design of active CDR-derived peptides. The Journal of Immunology, 2013, 190: 000–000.

The high affinity and specificity of Abs for their cognate Ags, which allows them to block Ag activity or to mark them for destruction (1), are at the heart of immunity. To understand immunity, one needs, therefore, to first identify and characterize the molecular determinants that are essential for Ag recognition and binding. Abs are also an effective and popular tool in biotechnology and biomedicine (2). Over the past two decades, >30 Abs and Ab derivatives have been approved for therapeutic use by the Food and Drug Administration (3). Although Abs in full IgG format dominate existing Ab-based technologies, a shift toward the study of smaller Abs fragments has been reported (4, 5). Because of their potential increased accessibility to epitopes and simpler delivery, smaller Ab fragments that retain specificity to the Ag may be better drugs. Such fragments have been shown to have improved pharmacokinetics as well as tissue and tumor penetration (5, 6). Understanding the contribution of the different Ab components to Ag recognition and binding may hold the key for designing such fragments.

Two Ab fragments, Fab and Fc, can be produced by papain enzymatic digestion (7). Fab is the Ag-binding component, and Fc is responsible for the induced effector functions. Single-chain fragment variable (scFv) is composed of a H chain variable domain (VH) and a L chain variable domain (VL), stabilized by a flexible linker (8). Although Fab and scFv cannot induce effector functions (e.g., recruit cellular response), they usually maintain specific Ag-binding capabilities (9) and may neutralize the target. In some cases, it has been shown that the H chain (10) or L chains (11) maintain Ag-binding specificity even when expressed on their own. Ward et al. (12) cloned a library of VH genes from spleen genomic DNA of immunized mice and isolated two VH fragments with Ag-binding affinities of $K_D$ = 12–27 nM. Such VH or VL fragments with binding activity are often dubbed single-domain Abs. Further analysis has led to the isolation of additional VH (13) and VL (14, 15) fragments capable of binding Ag individually. Yet, in many other cases, reports have been made of sticky behavior, low solubility (12), and reduced Ag-binding activity (13, 16) of such fragments. The introduction of Fab and scFv and the development of single-domain Abs demonstrated that different components of the Ab may bind the Ag independent of its other components. Thus, Abs may be considered modular proteins, comprising several elements that can bind the Ag on their own.

The fragmentation of the chains (VH or VL) might provide another level of modularity. Several studies have shown that linear peptides containing one or more of the CDRs retain Ag specificity and bind it even as peptides (17–24). Typically, the affinity of such peptides was in the high micromolar range, but, given their specificity, it has been shown that with relatively minor modifications they could become strong binders (22, 25). However, other studies concluded that such CDR-derived peptides lose the ability to bind the Ag (26, 27). Attempts to mimic the structure the CDR loop adopts in the context of the full Ab by adding a disulfide bond at the peptide edges has been suggested as a solution to this problem (21, 28, 29). In another study, stable peptides were produced by creating a peptide dimer and adding a single cysteine residue to one of the peptide termini on each peptide (30), or by joining two CDRs (31). The use of computational tools has also been explored for the design of Ag-binding peptides and to predict the strength of the peptide–protein interactions (25). In one case, peptide attached to a toxin was shown to inhibit tumor growth (31). These attempts to generate Ag-binding peptides are based on the idea that the interface itself may be modular, with some of its components capable of binding the Ag on their own. However, as

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The online version of this article contains supplemental material.

Abbreviations used in this article: HEL, hen egg white lysozyme; LDL, low-density lipoprotein; PDB, Protein Data Bank; RU, resonance unit; scFv, single-chain fragment variable; SPR, surface plasmon resonance; TFA, trifluoroacetic acid; VH, H chain variable domain; VL, L chain variable domain.

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mentioned earlier, attempts to scan Abs in search of peptides that maintain Ag binding have sometimes failed to identify such peptides (26, 27). It is widely assumed that the most important CDR for Ag binding is the third CDR of the H chain, CDRH3. Hence, many attempts to identify CDR-derived peptides that retain the specificity to the Ag focused on CDRH3. However, such efforts have succeeded in only a handful of cases. Currently, no way is available for identifying such peptides a priori or even to determine in advance whether they exist in a given Ab.

In this study, we show that CDRs that maintain Ag binding as peptides possess unique characteristics. First, compared with other CDRs, these CDRs are more instrumental to Ag binding. Second, they tend to interact with areas on the Ab that do not contact other CDRs. Computational analysis of the three-dimensional structure of the Ab–Ag complex that is based on these principles can distinguish between CDRs that were shown experimentally to bind the Ag on their own and CDRs that do not bind the Ag outside the context of the folded Ab. We experimentally screened Ab peptides and assessed their Ag binding. Because of the broad structural and functional information available, the interaction between HyHEL-10 scFv and hen egg white lysozyme (HEL) was chosen as a model (32, 33). The results of this experimental screening were in agreement with the computational ranking of the Ab CDRs. We further examined our hypothesis by analyzing the complex of proprotein convertase subtilisin–kinin type 9 (PCSK9) and 1D05 Ab, and then demonstrating experimentally that the predicted peptide indeed binds the Ag. In addition, we analyzed three Ab–Ag complexes for which a CDR-derived peptide was previously found and showed that in all three cases we could identify computationally the CDRs that binds the Ag.

Our results provide insight into the principles that guide antigenic interactions. Studies of the protein–protein interfaces indicate that most of the binding energy comes from very few residues (one or two on each side of the interface) (34). However, in Ab–Ag interfaces several CDRs may make significant contribution. Simple computational analysis may identify these CDRs and assist in designing potent Ag-binding peptides.

Materials and Methods

Computational and structural analysis

Crystal structures [Protein Data Bank (PDB) entries: 3C09 (35), 1N8Z (36), 2NY7 (37), 2DQJ (38), and 2XTJ (39)] of five different Ab–Ag complexes (matuzumab–epidermal growth factor receptor, Herceptin–HER2, b12-gp120, HyHEL-10–HEL, and 1D05–PCSK9, respectively) were downloaded from the PDB (40). The six CDRs were identified using the Kabat scheme (41). As discussed in Ref. 42, two residues were considered to be in contact if the distance between at least one nonhydrogen atom of each was \( \leq 5\AA \). For each CDR, Ag-contacting residues that fall within the loop of that CDR were also included. The number of residues in a CDR that are in contact with the Ag and the number of residues in the Ag that are in contact with the CDR were counted. Their sum was defined as the “number of residues in contact.” The number of potential H bonds, salt-bridges, and \( \pi-\pi \) and cation–\( \pi \) interactions between each of the CDRs and the Ag were calculated using the Discovery Studio Visualizer (3rd ed.; Accelrys, San Diego, CA). Their sum was defined as the “number of specific interactions.” An “independent residue” is that of an Ag residue in contact with residues belonging to only one CDR. An “integrated residue” is an Ag residue in contact with at least three CDRs. The percentage of independent or integrated residues for a given CDR was calculated from the total number of Ag residues in contact with that CDR.

The effect that mutating each CDR residue to Ala had on binding (in terms of \( \Delta G \)) was calculated using FoldX (43). First, each PDB structure was optimized using the FoldX RepairPDB function. Then, CDR amino acids were mutated to Ala, using the BuildModel function that generated mutants and their corresponding wild-type structure models. The AnalyzeComplex function was used to calculate the binding \( \Delta G \) of each model. “Calculated \( \Delta G \)” for each mutant was then computed by subtracting the wild-type calculated \( \Delta G \) value from the mutant calculated \( \Delta G \) value. The “degree of buriedness” was calculated using the DrugScore-PPI (44). The calculated \( \Delta G \) and the “degree of buriedness” of a CDR were considered the sum over its residues.

For each criterion of each Ab–Ag complex, values were normalized and scored according to their quartiles: 4 points for values within the top 25% of the scores, 1 for the values within the lowest 25%. The “total score” of a given CDR is the sum of the scores over its criteria. All indicated values are rounded to the second decimal point. The values of all criteria and the “total score” of each complex are provided in Table 1.

Cloning

Eleven Ab fragments were amplified from a plasmid encoding the HyHEL-10 scFv, kindly received from Gregory Winter’s laboratory in Cambridge, U.K. Primers used for the amplification contained EcoRI and NotI restriction sites. pGEX-4t-1 was used as an expression system to produce peptides fused to GST in their N terminus.

Peptide production

*Escherichia coli* (BI-21 DE3 strain) cells were transformed with a peptide-encoding vector. Bacteria were grown in liquid Luria–Bertani media at 37°C until it reached 0.6 OD. Then 1 mM isopropyl \( \beta \)-thiogalactoside was added, and the culture was grown for 3 more h at 37°C (except for H1-14 and H2-14, which were grown for an additional 18 h at 16°C). Cells were harvested and resuspended in PBS, 0.05% Triton, complete protease inhibitor (Roche). GST-fused peptides were purified by GST-binding beads according to the manufacturer’s protocol (Novagen). GST-fused peptides were eluted with 150 mM NaCl, and 10 mM glutathione reduced (Sigma-Aldrich), pH 7.5. Glyceraldehyde was added to 5% before freezing. Concentrations of GST and GST-fused CDR-derived peptides were measured by nanodrop in \( \lambda_{280} \) with an extinction coefficient of 47,000 \( \times \) 10^{-19} \text{cm}^{-1} \text{M}^{-1}.

**ELISA**

ELISA plates were coated with 0.4 \( \mu \)g Ag diluted in PBS at room temperature for 16 h. All subsequent steps were done at room temperature. Plates were washed three times with PBS-Tween (1:2000) between each step. Blocking was done with 1% BSA in PBS-Tween for 2 h. GST-fused peptides were diluted to 16 \( \mu \)M in reagent diluent (0.1% BSA. 0.05% Tween, 20 mM Tris, and 150 mM NaCl, pH 7.4) and applied to the plates for 2 h. GST-fused peptides were detected with 1:500 anti-GST HRP-conjugated mAb (sc-138; Santa Cruz Biotechnology). The plates were developed using chromogenic HRP substrate TMB, and color development was terminated with \( \text{H}_2\text{SO}_4 \). Synergy4 Reader (BioTek) was used to read plate values for \( \lambda_{450} \) nm and \( \lambda_{540} \) nm. A 540 nm was subtracted from A450 nm signal.

**Synthesis of cyclized peptide**

HyHEL-10-H2 and HyHEL-10-L3 were synthesized by Sigma-Aldrich. Two CYS residues were added to both N and C termini of CDR sequence to produce CVYSVSYGSTYC and QSNSWYPYTC, respectively. Thus, peptides included Ab positions (PDB numbering) 50–58 of the VH domain and 90–97 of the VL domain, respectively. The terminal CYS residues formed a disulfide bond, resulting in a cyclized peptide. Both peptides were biotinylated at the N terminus with e-amino-n-hexanoic acid or amino-3′-dioxoacetic acid linkers, respectively. The purity percentages of HyHEL-10-L3 and HyHEL-10-H2 were \( \geq 95\% \) and \( \geq 85\% \), respectively. 1D05-H3 and 1D05-L2 were synthesized by Biomatik and in house, respectively. Two CYS residues were also added to both N and C termini of CDR sequence to produce CYEIQIGRYGMNVYYLMC and CLIYNGSTLC, respectively. Thus, peptides included Ab positions (PDB numbering) 100–108 of the VH domain and 47–54 of the VL domain, respectively. The terminal CYS residues formed a disulfide bond, resulting in a cyclized peptide. Both peptides were biotinylated at the N terminus with e-amino-3′-dioxoacetic acid linker, respectively. The purity percentages of 1D05-H3 and 1D05-L3 were \( \geq 93\% \) and \( \geq 92\% \), respectively. 1D05-L2 peptide was synthesized as described further on.

Protected amino acids, BOP, HOBT, and chlorotrityl resin were purchased from GL Biochem (Shanghai, China). fmoc-amino-3′-dioxoacetic acid and biotin were purchased from Sigma-Aldrich. All solvents were analytically pure grade and were used without further purification. Analytical HPLC was performed on a Waters Gradient System equipped with a 717 Plus Autosampler, a Waters 600 Intelligent Pump, and a Waters 996 Photodiode Array Detector; the system was piloted with Millennium software and Waters. Selecting wavelength for two wavelengths (360 and 254 nm). Mobile phases were (A) \( \text{H}_2\text{O} \) [0.1% trifluoroacetic acid (TFA)] and (B) MeCN (0.08% TFA). The separation condition for analysis was as follows: Column Merck Chromolith Performance
Table I. Criteria for evaluation of the potential of CDRs to bind the Ag as peptides

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<tr>
<th>Ab name</th>
<th>PDB ID</th>
<th>CDR</th>
<th>Calculated ΔΔG (kcal/mol)</th>
<th>Specific Interactions</th>
<th>Residues in Contact</th>
<th>Degree of Buriedness</th>
<th>Independent Residues (%)</th>
<th>Integrated Residues (%)</th>
<th>Total Score</th>
</tr>
</thead>
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*HIS-mediated salt-bridge.
shown). Binding curves are the subtraction of channel one response from channel 2 response. The sonogram baseline was adjusted to 0 RU. Binding curves were exported to Excel.

Results

Ranking of CDRs according to their potential to independently bind the Ag

To demonstrate that our computational scheme can identify CDRs capable of binding the Ag, we performed a literature search for CDR-derived peptides that come from an Ab–Ag complex with an experimentally determined three-dimensional structure. We found three such examples. CDRH3 of anti-HER2 Ab [Herceptin; PDB ID: 1N8Z (36)] was used as a template to design a peptide mimic that maintains the HER2 inhibitory function (22), as well as CDRH3 of b12 Ab against HIV-1 gp120 [PDB ID: 2NY7 (37)], which was grafted into human mucin MUC1. The new protein, dubbed mucibody, was shown to bind HIV-1 gp120 and inhibit the gp120–CD4 interaction (50). In addition, the CDRH3 of matuzumab with a single substitution from ALA to ASP also has been shown to recognize the epidermal growth factor receptor (51). To explore the power of this approach beyond already known examples, we predicted Ag-binding CDR-derived peptides for two Ab–Ag complexes, and tested these predictions experimentally.

Our first model system was anti-HEL HyHEL-10 [PDB ID: 2DQJ (38)], which is a well-studied model of Ab–Ag interaction. The second model was the anti-PCSK9 Ab 1D05 (39). PCSK9 was shown to recognize the epidermal growth factor receptor (51). To examine which HyHEL-10 CDRs can bind the Ag individually, we isolated 11 CDR-derived peptides that contained one CDR flanked by 4–15 aa on each side, as described in Table II. The peptides included a variable number of flanking residues to stabilize the CDR loops. On the basis of the computational analysis, two additional cyclized peptides were selected (see below). Noncyclized peptides were fused to GST and purified from the bacterial cytoplasmic fraction by affinity chromatography based on glutathione Sepharose (Supplemental Fig. 2).

As seen in Table I and in Fig. 1, the ranking for independent binding of the same CDRs (e.g., H1) in different Abs may differ considerably. For Herceptin, CDRs H2, H3, and L1 had the highest scores, whereas for HyHEL-10 the top ranking was for CDRs H1, H2, and L1. For b12, CDRH3 ranked first, followed by CDRs H1 and H2. For both matuzumab and 1D05, CDRs H2 and H3 had the highest ranking. Moreover, it seems that for each Ab–Ag complex a clear separation exists between highly ranked CDRs and poorly ranked ones, where two or three CDRs stand out as the most probable to bind the Ag as peptides.

The ranking of CDRs is in agreement with the experimental design of previously described matuzumab, Herceptin, and b12 CDR-derived peptides (which were all based on CDRH3). Matuzumab CDRH3 had the highest total score and led in five of six analysis parameters. Herceptin CDRH3 ranked only two points below the highest ranking CDRH2, and b12 CDRH3 ranked as top candidate for independent binding in five of the six parameters. The experimental results for HyHEL-10 and 1D05 CDR-derived peptides, which confirmed the predictions, will be discussed further on.

HyHEL-10 CDRL1- and CDRH1-derived peptides maintained HEL binding

To examine which HyHEL-10 CDRs can bind the Ag individually, we isolated 11 CDR-derived peptides that contained one CDR flanked by 4–15 aa on each side, as described in Table II. The peptides included a variable number of flanking residues to stabilize the CDR loops. On the basis of the computational analysis, two additional cyclized peptides were selected (see below). Noncyclized peptides were fused to GST and purified from the bacterial cytoplasmic fraction by affinity chromatography based on glutathione Sepharose (Supplemental Fig. 2). Fig. 2 shows the results of the ELISA screen for these GST-fused CDR-derived peptides. Two peptides, H1-9 and L1-10, retained specificity to HEL. Compared with the background levels of OD observed for GST, the average of the OD values for H1-9

Table II. HyHEL-10 CDR-derived peptides

<table>
<thead>
<tr>
<th>Fragment Name</th>
<th>CDR</th>
<th>Number of Residues from Each Side of CDR</th>
<th>Fragment Length (Residues)</th>
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<tbody>
<tr>
<td>H1-14</td>
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<td>14</td>
<td>33</td>
</tr>
<tr>
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<td>H1</td>
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<tr>
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<tr>
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<td>H3</td>
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<tr>
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<tr>
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<tr>
<td>L3-10</td>
<td>L3</td>
<td>10</td>
<td>29</td>
</tr>
</tbody>
</table>

Fragment length includes both CDR residues and the flanking residues. Fragment length does not include the fused GST.
9 showed clear binding. Similarly, L1-10 showed average value that was more than twice that of GST alone. For CDR-derived peptides that did not show clear negative results (i.e., OD values similar to that of GST) in duplicates, we repeated the analysis up to 6 times (solid bars) to establish the difference or lack thereof.

To further validate the specificity of these peptides, BSA-coated wells were used as negative control (Fig. 2B). Both H1-9 and L1-10 showed binding to HEL, but not to BSA-coated wells.

**Structural design of HyHEL-10 cyclized CDRH2 resulted in Ag binding**

For HyHEL-10, two of the three top-ranked CDRs (Table I) were experimentally verified (CDRH1 and CDRL1). However, although CDRH2 was ranked as the CDR with the highest contribution to Ag binding, it showed no binding in this experiment. We hypothesized that this is because CDRH2 peptides did not adopt the right conformation required for Ag binding. As shown in Fig. 3A, four residues of CDRH2 are involved in specific interactions with the Ag: TYR50 of CDRH2 forms two H bonds with ARG21 of the Ag, SER54 of CDRH2 also has two H bonds with ASP101 of the Ag, SER56 of CDRH2 has an H bond with the NH backbone of GLY102 of the Ag, and TYR58 of CDRH2 has a cation–π interaction with ARG21 of the Ag. However, these four residues, which are a part of a nine residues loop (TYR50–TYR58), are not consecutive in sequence. Thus, to retain all these interactions simultaneously, CDRH2 must adopt a specific conformation. In contrast, in CDRH1 and CDRL1, only two consecutive residues are involved in specific interactions with the Ag (Fig. 3B, 3C), and therefore, these CDRs are more likely to adopt a conformation that allows Ag binding even as peptides. Limiting the conformational space of CDRH2 by cyclization may, therefore, increase the chance that it will adopt a conformation that allows the interaction of all four residues with the Ag, and thus may result in binding.

To test this hypothesis, we synthesized CDRH2 cyclized peptide (HyHEL-10-H2). Cyclization was performed by allowing a disulfide bond between two CYS residues that were added to both termini of the peptide. CYS residues were added before TYR50 and after TYR58. The π–π interaction between the phenyl groups of these two TYRs and the two H bonds between their backbones may further stabilize the right conformation of the loop. HyHEL-10 CDRL3 peptide (HyHEL-10-L3) was cyclized as well for negative control. Although technical issues prevented the evaluation of HEL binding by GST-fused peptides using SPR, the synthetic cyclized peptides are not GST fused, and therefore their binding could be examined using SPR. Binding curves with association and dissociation phases were observed for HyHEL-10-H2, whereas only bulk response was observed for HyHEL-10-L3 (Fig. 4A, Supplemental Fig. 1). In addition, steady-state affinity analysis was performed, and a hyperbolic binding plot for

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**FIGURE 2.** Binding of HyHEL-10 CDR-derived peptides. ELISA was used as a binding screen (A). Percent of the peptide signal is the percent of CDR-derived peptide OD (450–540 nm) values from GST OD (450–540 nm) values. Peptides concentration is 16 μM. Dashed columns are the average of duplicate wells from one plate. For solid columns, error bars represent the SE, and columns represent the average of duplicate wells from two plates (three plates for L1-10 and H1-9). BSA-coated wells were used for estimating binding specificity (B). HEL-coated wells (black columns) and BSA-coated wells (white columns) were blocked and incubated with 16 μM of H1-9, L1-10, or GST as a negative control. Values are the average of duplicate wells.

**FIGURE 3.** Conformation of three CDRs of HyHEL-10 predicted to bind as peptides: (A) CDRH2, (B) CDRH1, and (C) CDRL1. The Ag is shown in surface representation, colored gray. The CDRs are green, in backbone representation. Amino acids involved in specific interactions with the Ag are presented as orange sticks, and only polar hydrogens are shown. Image was generated from PDB ID: 2DQJ, using Discovery Studio Visualizer (3rd ed.; Accelrys, San Diego, CA).

**FIGURE 4.** Binding of cyclized CDR-derived peptides to HEL. SPR was used to test HEL binding of HyHEL-10-H2 and HyHEL-10-L3. Binding curves (A) were obtained with Biacore T100 equipped with a CM5 biosensor chip (see Materials and Methods for more details) for 38 μM (dark gray), 174.2 μM (light gray) of HyHEL-10-L3 and 38 μM (low black curve), 174.2 μM (high black curve) of HyHEL-10-H2. (B) Steady-state analysis for HyHEL-10-H2 (black) and for HyHEL-10-L3 (gray).
HyHEL-10-H2 was observed (Fig. 4B). Thus, although CDRH2 did not appear as an Ag binder in the form of a linear peptide, it did bind the Ag as a cyclized peptide. This result is in agreement with the computational CDR ranking (Table I) and with the hypothesis regarding the correct conformation required for CDRH2 binding (Fig. 3).

**Cyclized peptide of the top-ranking CDRH3 of 1D05 maintained Ag binding**

In addition to experimental validation of the predicted CDR-derived peptides for HyHEL-10 and the agreement between the predictions and the previously published results for Herceptin, b12, and matuzumab, we also analyzed the 1D05–PCSK9 complex. 1D05 CDRH3 was ranked at the top, with the highest ΔΔG compared with all the CDRs in all five Abs we analyzed. Four nonconsecutive residues of CDRH3 are involved in five specific interactions with PCSK9: one salt-bridge and four H bonds (PDB ID: 2XTJ). Hence, we expected that peptide cyclization will allow CDRH3 peptide to adopt a binding conformation. For the design of the cyclized peptide 1D05-H3, cyclization was performed by allowing a disulfide bond between two CYS residues that were added to both termini. CYS residues were added before TYR100 and after MET115. As shown in Fig. 5, the loop between these two residues is stabilized by five intramolecular H bonds, and the loop contains all five specific interactions of CDRH3 with PCSK9. CDRL2 of 1D05, which received the lowest “total score,” was cyclized as well as a negative control (1D05-L2).

SPR experiments resulted in binding curves for injections of 0.156–3.5 μM of 1D05-H3, and association and dissociation phases are observed (Fig. 6A). For 1D05-L2, injections of the same concentrations resulted only in bulk response (Fig. 6B). Thus, as was predicted by the computational CDR ranking, 1D05 CDRL2-derived cyclized peptide retained Ag binding, whereas CDRL2-derived cyclized peptide did not.

**Discussion**

Previous studies (17–19, 21, 28–30, 53) have obtained Ab fragments as small as a single CDR that maintain specificity for the Ag. Relatively minor modifications could increase their affinity and turn these fragments into potent binders. Nevertheless, in these studies, the identification of the peptides is mostly based on incidental evidence and seldom on comprehensive screening. In addition, some attempts to obtain such fragments have failed (26, 27). The first bottleneck to obtain such fragments is choosing a potent CDR for Ag binding, and the second one is the design of a peptide with a conformation allowing peptide–Ag interaction. We provide an analytical framework for identifying potent CDRs and also address to the rationale for their Ag binding.

The computational scoring attempted to identify independent CDRs by assessing CDR binding contribution and interface modularity. Four of the criteria—calculated ΔΔG, number of specific interactions, number of residues in contact, and degree of buriedness—are direct assessments of the contribution of each CDR to the affinity of the Ab toward its Ag. The two remaining criteria, percent of independent residues and percent of integrated residues, evaluate to what extent the interaction between a CDR and the Ag does not involve other CDRs. Although the contributions of the different criteria are probably not identical, in the current study we did not integrate them into a final score using different weights. Future analysis is required to explore the relative contribution of each of these factors.

Structure-based strategy was applied to address the issue of peptide conformation. We used the Ab–Ag complex structures to design cyclized peptides that retain all residues that involve specific interactions and may confer the original CDR conformation.
Peptides were cyclized by adding a disulfide bond to minimize the conformational space, as was also described previously (21, 28).

CDRH3 is often considered to have a special significance for Ag recognition (54–56). Thus, many previous efforts, including the three discussed examples in this study (22, 50, 51), focused on CDRH3 for the development of CDR-derived peptides. However, we found that in some cases other CDRs may be more important and that there are Abs in which CDRH3 is not a promising starting point for an Ag-binding peptide. For example, for HyHEL-10, CDRH3 ranked at the bottom. For matuzumab and Herceptin, we found that although CDRH3 scored high, other CDRs ranked as high. Indeed, many CDR-derived peptides are not based on CDRH3, such as the following: A peptide derived from CDRH2 of T15 Ab against phosphorylcholine was shown to inhibit its activity (18); cyclized CDRH1 of 8D4 inhibited NS3 protease activity (23); and part of CDRL1 and FW1 of 16D7 Ab was shown to bind HP2/6 Ab (57). In all five Ab–Ag complexes we analyzed, CDRs L2 and L3 did not rank as likely to bind the Ag on their own. Although CDRs L3 and L2 are considered to have average or low contribution to Ag binding (55, 58), some studies demonstrated that they can serve as a basis for Ag-binding peptides (30, 59, 60). Thus, the identification of potent CDR-derived peptide for each Ab–Ag complex should not rely on the concept that some CDRs are more important than others, but on rational ranking of the CDRs in a given Ab–Ag complex.

Although CDR-derived peptides in the current form might exhibit low affinity and specificity, they could serve as precursors for improved peptides that will be developed by chemical modifications and affinity maturation techniques. Such improved peptides may be used in therapeutic or diagnostic application. In comparison with full IgG, these peptides may display advanced tissue and tumor penetration, reduce cost, and possibly decrease immunogenicity. For example, various analogs of CDRH3 of Herceptin were designed (mainly by addition of a polar group, mutation of MET to LYS, or introduction of D-isomers) (61). Some of them had improved affinity, inhibition activity, or solubility. Another study has shown that a toxin-fused drug candidate composed of CDRH1 and CDRL3 fused through VH framework region 2 of an Ab against gp350/220 (EBV envelope protein) has superior capacity to penetrate tumor and inhibit tumor growth (31). Such studies highlight the applicable relevance of CDR-derived peptides.

For HyHEL-10, we scanned different components of the Ab. Computational analysis suggested that three of the HyHEL-10 CDRs—namely, H1, L1, and H2—have binding capacities independent of other parts of the Ab. However, CDRH2 binds the Ag in a specific conformation that may require cyclization to stabilize it. This hypothesis was verified by the experimental results. Interestingly, longer flanking sequences around the CDRs were not always a guarantee for better binding, as H1-9 and L1-10 did bind, whereas H1-14 and L1-15 did not. The optimal number of flanking residues is possibly different for each CDR in each Ab. A thorough analysis of Ab–Ag complexes (experimentally or by computational methods like molecular dynamics) may assist in designing CDR-derived peptides with an optimal number of flanking residues.

On the basis of agreement between the predictions and experimental results in the current study and in previous studies (22, 50, 51), we suggest that simple computational analysis may allow for discrimination between CDRs that may serve as a basis for CDR-derived peptides and those that may not.

In conclusion, this study provides additional support for the Ab modularity hypothesis by presenting smaller Ab components that retain Ag binding and specificity as well as a computational framework for identifying such peptides. Additional analysis may allow further characterization of the CDR properties responsible for Ag binding and may lead the way to increase the specificity and affinity of such peptides.

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**Disclosures**

The authors have no financial conflicts of interest.

**References**


