Induced Fit of an Epitope Peptide to a Monoclonal Antibody Probed with a Novel Parallel Surface Plasmon Resonance Assay*

Received for publication, September 16, 2004, and in revised form, November 17, 2004
Published, JBC Papers in Press, November 18, 2004, DOI 10.1074/jbc.M410687200

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Class II major histocompatibility complex proteins bind peptides for presentation to T-cells as part of the immune response process. Monoclonal antibody MEM-265 recognizes the peptide-free conformation of the major histocompatibility complex class II protein HLA-DR1 through specific binding to an epitope contained between residues 50–67 of the β-chain. In previous work using alanine scanning (1), we identified residues Leu-53, Asp-57, Tyr-60, Trp-61, Ser-63, and Leu-67 as essential for specific recognition by MEM-265. The spacing of these residues approximates a 3.5-residue repeat, suggesting that MEM-265 may recognize the epitope in an α-helical conformation. In the folded, peptide-loaded DR1 structure, the β-chain residues 50–67 contain a kinked α-helical segment spanning Glu-52–Ser-63 (2). However, the conformation of this segment in the peptide-free form is unknown. We have used a new surface plasmon resonance approach in a SpotMatrix format to compare the kinetic rates and affinities for 18 alanine scanning mutants comprising epitope residues 50–67. In addition to the six essential residues described previously, we found two additional residues, Glu-52 and Gln-64, that contribute by enhancing MEM-265 binding. By contrast, mutation of either Gly-54 or Pro-56 to an alanine actually improved binding to MEM-265. In essentially all cases peptide substitutions that either improve or reduce MEM-265 recognition could be traced to differences in the dissociation rate (kₐ). The kinetic details of the present study support the presence of a structural component in the antigenic epitope recognized by MEM-265 in the peptide-free form of major histocompatibility complex II DR1 β-chain.

Research on factors influencing molecular recognition is an area of vibrant activity. The molecular basis of specificity for antigen-antibody recognition can involve numerous factors and is not limited to the primary amino acid sequence of the antigenic epitope. The epitope can have a complex structural component in the context of the entire tertiary structure of the antigen. In such a case, an analogous linear variant of the original antigen, for instance, a short peptide, would not be expected to retain much structural information, particularly in the absence of any disulfide bridges. However, antibodies that can recognize short peptides that appear to adopt a defined conformation have previously been described (3, 4).

MEM-265 is a mouse monoclonal antibody that recognizes the empty conformation of the human class II MHC protein HLA-DR1. Although the antibody was raised against the denatured β subunit, it appears to recognize a conformational epitope present in the native α-β heterodimer, which becomes unavailable upon peptide binding (1). Preliminary characterization has mapped the epitope to residues 50–67 of the β subunit, with residues Leu-53, Asp-57, Tyr-60, Trp-61, Ser-63, and Leu-67 being essential for binding. To assess the individual contributions of nonessential residues to binding, we used SpotMatrix SPR† to obtain a comparative kinetic analysis for alanine mutants spanning residues 50–67 of the epitope.

The application of SpotMatrix SPR for the affinity characterization of monoclonal antibodies in the context of epitope mapping is described here. In this approach, biotinylated peptides are spotted at a range of concentrations onto a specific capture surface to be enclosed into a single flow cell. The entire SpotMatrix of peptides can then be simultaneously interrogated against a specific analyte in a single experiment by surface plasmon resonance to obtain side-by-side comparative binding kinetics (see “Experimental Procedures”). Peptide epitope mapping by imaging SPR has been described previously in a lower density format for the monoclonal anti-FLAG M2 antibody and FLAG epitope (5). In the present study, we characterize the monoclonal antibody, MEM-265, raised against denatured MHC II DR1 β-chain (1). Relative binding and kinetic rate differences between parent and mutant peptides were simultaneously assessed using the SpotMatrix SPR technology. Certain unique structure-activity inferences emerged that provide further support for the hypothesis that MEM-265 recognizes an epitope that is contained within the secondary structure of an α-helix.

EXPERIMENTAL PROCEDURES

Peptide Synthesis and Purification—Peptides carrying N-terminal biotin tags were prepared as described previously (1). Briefly, the peptides were synthesized in-house using standard N-(9-fluorenylmethoxycarbonyl) (Fmoc) solid phase chemistry and were biotinylated while still bound to the solid phase, using biotin-LC-LC-NHS ester (succinimidyl-6′-(brominamido)-6′-hexanamido hexanoate; Pierce) to modify the N-terminal amino group, before conventional deprotection and isolation by reverse-
phase HPLC. Purity was determined by analytical HPLC and mass spectrometry. Table 1 lists the peptides used for this study.

Development of Monoclonal Antibodies—MEM-265 antibody was purified by protein A affinity chromatography from the culture supernatants of hybridoma cells grown in serum-free medium (Invitrogen) in culture flasks or hollow fiber bioreactors. The MEM-265 hybridoma was originally prepared after immunization of mice with purified, insoluble DR1 β-chain (DRB1*0101) expressed in Escherichia coli inclusion bodies and screening of hybridomas for the ability to distinguish empty DR1 from hemagglutinin peptide loaded DR1 using enzyme-linked immunosorbent assay. MEM-265 is one of four similar antibodies thus identified (1).

**Binding Kinetics by SpotMatrix Surface Plasmon Resonance—**Binding kinetics were measured using a FLEX CHIP Kinetic Analysis System (HTS Biosystems, East Hartford, CT) that uses grating-coupled surface plasmon resonance technology for parallel measurement of real time binding kinetics between unlabeled analytes and up to 400 target biomolecules immobilized (spotted) on an affinity chip to form a Spot-Matrix. Surface plasmon resonance occurs when light, under particular conditions, is reflected off of certain thin metal films such as gold. When the energy state of the incoming photons matches the energy modes of the delocalized metal surface electrons (the plasmons), energy is transferred to the surface. This energy transfer is recorded as a decrease in the intensity of the reflected light. The incident light angle at which this energy transfer is most efficient, the SPR angle, is very sensitive to the intensity of the reflected light. The incident light angle at which this energy transfer is recorded as a decrease in the energy state of the incoming photons matches the energy modes of the support substrate and thickness of the gold layer. In the grating-coupled configuration, a fine grating on the chip surface provides optical coupling and allows imaging of the entire surface at once, enabling simultaneous real time binding analysis at every spot on the surface. Here, incident light hits the gold layer from the top and through the biomolecular layer, avoiding the stringent need for an optical quality support substrate and specific gold layer thickness. The chips are disposable.

**SPR Biosensor Optics**

In the Kretschmann prism configuration, incident light hits the gold layer on the opposite side of the immobilized biomolecules, posing strict requirements for the optical properties of the support substrate and thickness of the gold layer. In the grating-coupled configuration, a fine grating on the chip surface provides optical coupling and allows imaging of the entire surface at once, enabling simultaneous real time binding analysis at every spot on the surface. Here, incident light hits the gold layer from the top and through the biomolecular layer, avoiding the stringent need for an optical quality support substrate and specific gold layer thickness. The chips are disposable.

**Table 1**

*Sequence of wild-type and mutant peptides*

| ID | Peptide sequence |
|----|------------------|
| WT | V T E L G R P D A E Y W N S Q K D L |
| V50A | A T E L G R P D A E Y W N S Q K D L |
| T51A | V A L E G R P D A E Y W N S Q K D L |
| E52A | V T E A L G R P D A E Y W N S Q K D L |
| L53A | V T E L A G R P D A E Y W N S Q K D L |
| G54A | V T E L A R P D A E Y W N S Q K D L |
| R55A | V T E L A R P D A E Y W N S Q K D L |
| P56A | V T E L A R P A E Y W N S Q K D L |
| E57A | V T E L A R P A E Y W N S Q K D L |
| E58A | V T E L A R P A E Y W N S Q K D L |
| Y60A | V T E L P D A R A E Y W N S Q K D L |
| W61A | V T E L P D A R A E Y W N S Q K D L |
| N62A | V T E L P D A R A E Y W A S Q K D L |
| S63A | V T E L P G D A R A E Y W A S Q K D L |
| Q64A | V T E L P G D A R A E Y W N S A K D L |
| K65A | V T E L P G D A R A E Y W N S A D L |
| D66A | V T E L P G D A E Y W N S A D A L |
| L67A | V T E L P G D A E Y W N S A A D A L |

**Fig. 1. SPR configurations.** In the Kretschmann prism configuration, incident light hits the gold layer on the opposite side of the immobilized biomolecules, posing strict requirements for the optical properties of the support substrate and thickness of the gold layer. In the grating-coupled configuration, a fine grating on the chip surface provides optical coupling and allows imaging of the entire surface at once, enabling simultaneous real time binding analysis at every spot on the surface. Here, incident light hits the gold layer from the top and through the biomolecular layer, avoiding the stringent need for an optical quality support substrate and specific gold layer thickness. The chips are disposable.
Given that the amount of free ligand [B] at any particular time can be described as the amount of total ligand at the surface [B\text{total}] minus ligand bound with analyte [AB], [B] can be substituted by [B\text{total}] − [AB]. Furthermore, the SPR response signal, R, is proportional to complex [AB] by a certain constant, C, so that \( R = C[AB] \). When [B\text{total}] is fully bound by analyte, [B\text{total}] = [AB] and \( R = R_{\text{max}} \), the maximum SPR response signal. Substituting these equivalencies into the above equation gives rise to the following equation.

\[
\frac{d[R]}{dt} = k_{\text{on}}(R_{\text{max}} - R) \left[ \frac{k_{\text{off}}[A][C] + k_{\text{on}}R}{k_{\text{on}}[A][C] + k_{\text{off}}(R_{\text{max}} - R)} \right] - k_{\text{off}}R \quad (\text{Eq. 3})
\]

The analytical solutions for both association and dissociation phases (12), based on quasi-steady state approximation of the two-compartment model were employed in the algorithm, part of FLEX CHIP kinetic analysis system data analysis 4.1.3 software. The algorithm fits all of the affinity traces for a given peptide concurrently and reports a single \( k_{\text{on}} \) and \( k_{\text{off}} \) term for the entire group. The algorithm applies a correction for mass transport limited kinetics. The mass transport rate constant \( (k_{\text{m}}) \) and \( R_{\text{max}} \) values are fit independently for each curve in the group. The conditions used for the present analysis used low, subsaturating densities of spotted peptides (1–3 \( \mu \)M used, saturation at 10–20 \( \mu \)M) and sought to take into account initial dissociation rates to reduce the contribution of bivalent binding of the monoclonal antibody to the immobilized peptides. Residual (data fit) maps for all data points of the analyzed data sets defined the extent of error at different time points between experimental data and theoretical model. Association was evaluated for 30 min, and dissociation was evaluated over a period of 3–4 min to minimize any antibody rebinding effects because of the antibody bivalence. Plots of residuals and \( \chi^2 \) values were used to judge data quality. Residual plots depict the difference between experimental data and theoretical data for each time point of the binding event. The \( \chi^2 \) value represents the average of the squared residual values.

Peptide Structure Prediction—Secondary structural prediction for wild type and mutant peptides was performed using method 1 of the Secondary Structural Content Prediction (SSCP) (13, 14), version 2.0 software of Eisenhaber and Imperiale (www.bork.embl-heidelberg.de/SSCP/sspseq.html). Method 1 relies on the average amino acid composition of secondary structural elements in a defined set of proteins. SSCP predicts the tendency of a linear sequence in the context of a protein to assume a secondary structure. Secondary structural prediction was also performed using the prediction algorithm AGADIR (15) (www.embl-heidelberg.de/Services/serrano/agadir/agadir-start.html). AGADIR predicts the helical propensity of peptides free in solution.

RESULTS

Kinetic Analysis of Alanine Scan Epitope Peptides—Peptide D66A at nine concentrations of 1, 2, and 3 \( \mu \)M was spotted in triplicate forming a group of nine spots. In Fig. 2B, a graphic representation of the 12 \( \times 15 \) matrix highlights the relative positions of the nine spots forming the peptide group D66A. The affinity traces generated by this group upon binding MEM-265 (25 nM) are shown in Fig. 2C. During the hour-long chip equilibration, the first 60 min of the kinetic run, minimal drift was observed as noted by the flat base line. The three sets of triplicate affinity traces form three distinct groups with \( R_{\text{max}} \) values of \(-2\), 10, and 20 RCU for D66A peptide spotted concentrations of 1, 2, and 3 \( \mu \)M, respectively. The affinity traces
represents reference-corrected data where, for each time point, the average value of the four reference areas surrounding a peptide spot was subtracted from the RCU value for the particular spot. Reference areas are located in the spaces between peptide spots and correspond to surface areas where no sample has been spotted (not shown).

Kinetic analysis of the D66A peptide group began by autozeroing the nine affinity traces at the 57-min time point. The association period for analyte MEM-265 was from the 60-min time point to the 90-min time point, whereas the dissociation phase was analyzed from the 90-min time point to the 94-min time point. Affinity traces were globally fitted with mass transport correction, and the resulting theoretical traces are shown overlaid on the experimental affinity traces (Fig. 3A). The degree to which experimental and theoretical affinity traces correlate at each time point is shown in the residual plot (Fig. 3B). A tight correlation was observed, indicating that binding of MEM-265 to immobilized D66A peptide, monitored under this particular set of conditions, a simple 1:1 binding model was sufficient to describe the interaction. Kinetic rates and equilibrium constants were simultaneously and globally calculated for the D66A group affinity traces (Table II). Peptide spotted at higher concentrations (2–3 μM) required a smaller $k_{\text{on}}$ term, indicating that diffusion of analyte MEM-265 to the surface was more rate-limiting when peptide was spotted at higher concentrations.

As described above for D66A peptide group, a similar analysis was carried out for the rest of the peptide groups. High

### Table II

| Spot ID (concentration) | $k_{\text{on}} \times 10^4$ M$^{-1}$ s$^{-1}$ | $k_{\text{off}} \times 10^{-5}$ s$^{-1}$ | $K_D \times 10^{-9}$ M | $\chi^2$ |
|------------------------|----------------------------------|----------------------------------|------------------|--------|
| D66A (1 μM)             | 4.11                             | 1.78                             | 2.32             | 4.32   |

### Table III

| ID | $k_{\text{on}} \times 10^4$ M$^{-1}$ s$^{-1}$ | $k_{\text{off}} \times 10^{-5}$ s$^{-1}$ | $K_D \times 10^{-9}$ M | $\chi^2$ |
|----|----------------------------------|----------------------------------|------------------|--------|
| WT | 7.24                             | 5.75                             | 2.10             | 0.14–0.23 |
| V50A | 3.21                             | 15.10                            | 4.69             | 0.09–0.20 |
| T51A | 4.28                             | 15.80                            | 3.68             | 0.12–0.16 |
| E52A | 3.22                             | 42.90                            | 13.30            | 0.11–0.42 |
| L53A | NB                               | NB                               | NB               |         |
| G54A | 2.60                             | 0.89                             | 0.34             | 0.45–0.71 |
| R55A | 3.71                             | 7.72                             | 2.08             | 0.09–0.48 |
| F56A | 2.53                             | 1.62                             | 0.64             | 0.31–0.93 |
| D57A | NB                               | NB                               | NB               |         |
| E58A | 1.59                             | 1.89                             | 1.19             | 0.44–0.95 |
| Y60A | NB                               | NB                               | NB               |         |
| W61A | NB                               | NB                               | NB               |         |
| N62A | 1.68                             | 5.26                             | 3.13             | 0.25–0.81 |
| S63A | NB                               | NB                               | NB               |         |
| Q64A | 3.30                             | 36.30                            | 11.00            | 0.10–0.32 |
| K65A | 2.83                             | 3.47                             | 1.22             | 0.37–0.60 |
| D66A | 4.11                             | 17.80                            | 4.32             | 0.08–0.23 |
| L67A | NB                               | NB                               | NB               |         |

The kinetic parameters for the 18 peptides were obtained by globally fitting the data to a 1:1 model with mass transport correction. WB, the SPR binding signals obtained for W61A were too weak to provide reliable kinetic fits. NB, MEM-265 did not bind to L53A, D57A, Y60A, S63A, and L67A. WT, wild type.

### Fig. 4

Screening analysis for the 12 peptides that provided kinetic content. The panel depicts a log($k_{\text{on}}$) versus log($k_{\text{off}}$) for the 12 alanine scanning peptides that provide suitable affinity traces for kinetic evaluation. The diagonal lines represent $K_D$ values. Alanine scan mutagenesis for this set of peptides preferentially impacted $k_{\text{on}}$ rates.

SPR binding signals (10–30 RCU) were obtained for MEM-265 (25 nM) binding to peptides wild type, V50A, T51A, E52A, G54A, R55A, P56A, E59A, N62A, Q64A, K65A, and D66A. A weak SPR signal (3 RCU) was observed for MEM-265 binding to W61A. Under the experimental conditions used in this study, MEM-265 did not bind to L53A, D57A, Y60A, S63A, and L67A. These results are consistent with previous enzyme-linked immunosorbent assay results, which identified Leu-53, Asp-57, Tyr-60, Trp-61, Ser-63, and Leu-67 as constituting the most critical residues for MEM-265 recognition. The SPR results reported here were obtained by spotting the biotinylated peptides on a NeutrAvidin™ affinity chip. Results on a streptavidin affinity chip were similar and showed the same overall trends but somewhat lower RCU values (data not shown), indicating a very limited role, if any, for a particular surface biasing the present data set.

Alanine Substitution of Nonessential Epitope Residues Primarily Affects Off Rates—Kinetic constants were calculated for each mutant peptide, and the data were analyzed as described above. The results, summarized in Table III, show that although the measured $k_{\text{on}}$ rates for various peptides varied only 2.7-fold ranging from $1.59 \times 10^{6} \text{M}^{-1} \text{s}^{-1}$ to $4.29 \times 10^{6} \text{M}^{-1} \text{s}^{-1}$, the $k_{\text{off}}$ rates varied over a much larger 48-fold range between $0.89 \times 10^{-9} \text{s}^{-1}$ and $42.9 \times 10^{-9} \text{s}^{-1}$. The range of the log of $k_{\text{on}}$ and $k_{\text{off}}$ values for globally analyzed data sets corresponding to the various peptides are also listed in Table III. The quality of the fit was good with all of the values coming in below 1. To facilitate comparative analysis of kinetic parameters, $k_{\text{on}}$, $k_{\text{off}}$, and $K_D$ for a series of multiple ligand interactions with an analyte, we utilized log $k_{\text{on}}$ versus log $k_{\text{off}}$ plots, which also depict $K_D$ and are often used in this context.
regard (Fig. 4). For the 12 peptides carrying mutations on residues that were not essential for MEM-265 binding, the log $(k_{on})$ versus log $(k_{off})$ kinetic parameter plot clearly shows that the alanine scan mutations preferentially affect the dissociation rate without exhibiting much of an affect on the association rate. Mutations E52A and Q64A exhibited partial detrimental effects on MEM-265 binding, indicating that residues Glu-52 and Gln-64 are not critical for binding. Mutations G54A and P56A enhanced MEM-265 affinity relative to wild type. The dissociation rates of MEM-265 for E52A and Q64A relative to wild type increased 8-fold, whereas the dissociation rates of MEM-265 for G54A and P56A relative to wild type decreased six and 4-fold, respectively. In each case, the apparent association rates for all of these peptides were relatively unchanged, and the effects of mutation on the equilibrium dissociation constant, $K_D$, are essentially due to alterations of the dissociation rates. An overlay of MEM-265 affinity traces for E52A and P56A peptides spotted at comparable densities and eliciting similar $R_{max}$ values graphically illustrates that specificity is primarily due to dissociation rate differences (Fig. 5).

Correlation between Epitope Helical Propensities and Kinetic Parameters—G54A and P56A were the substitutions that provided the greatest increase in affinity and decrease in dissociation rate. Both glycine and proline within the context of an $\alpha$-helical segment are generally considered to be destabilizing (16). Substitution of these helical destabilizing residues with alanine, which is generally considered to be stabilizing for $\alpha$-helices, had an enhancing effect on the affinities of mutant peptides for MEM-265. To better ascertain the possible structural influence of alanine substitutions on kinetic parameters of MEM-265 binding, the degree of predicted helical propensity of the various peptides was plotted as a function of the log of the kinetic rates (Fig. 6). Two algorithms were used to predict the helical tendencies of the peptide set: SSCP (13, 14) (Fig. 6, A and B) and AGADIR (15) (Fig. 6, C and D). Whereas SSCP uses a protein set to make a prediction of secondary structure, AGADIR predicts the tendency of the peptide, free in solution, to be helical. In the present case, however, neither algorithm may adequately model

![Fig. 5](overlay_of_mem_265_affinity_traces_for_e52a_and_p56a.png)

**Fig. 5. Overlay of MEM-265 affinity traces for E52A and P56A.** Both E52A and P56A peptides were spotted at comparable densities. Upon interrogation with MEM-265, similar $R_{max}$ values were observed, indicating a similar amount of antibody bound to each of the two different spotted peptides. Association traces for the most part superimpose. Dissociation rate differences are the primary cause of specificity.

![Fig. 6](plots_of_helical_propensity_vs_log_kinetic_rates.png)

**Fig. 6. Plots of helical propensity versus log (kinetic rates).** A and B depict the percentage of $\alpha$-helical character predicted by SSCP for the mutant peptides as a function of the log of the kinetic rates. SSCP predicts helical propensity in the context of a protein sequence. Although helical propensity does not correlate with the association rate (A), a correlation between helical propensity and dissociation rates is observed for a number of the peptides, such as E52A, G54A, P56A, and Q64A (B). C and D depict the percentage of $\alpha$-helical character predicted by AGADIR for the mutant peptides as a function of the log of the kinetic rates. AGADIR predicts helical propensity of free peptides in solution. AGADIR predicted that helical propensity does not correlate with either the association rate (C) or dissociation rates (D).
and group 4 may affect MEM-265 dissociation rates through a more subtle indirect effect on the positioning of the critical group 1 residues. The residues in both group 2 and group 4 are located immediately adjacent to critical residues. Group 2 residues may assist by sterically positioning group 1 residues in conformationally restricted positions, which are favorable for MEM-265 binding. By the same reasoning, group 4 residues may exact a considerable conformational cost for their neighboring critical residues. Whereas glycine at position 54 allows for too much conformational freedom, proline at position 56 may restrict the conformational freedom of neighboring residues, such as Leu-53 (Fig. 7), to positions that would not be optimal for complete antigenic variant peptide-antibody recognition.

In the context of the fact that the critical residues of the MEM-265 epitope are spaced appropriately to reside on a contiguous face of an α-helix and of the fact that residues 50–67 in the folded MHC II DR1 β-chain structure are part of a helix, the kinetics provided for these mutations further support the possibility that MEM-265 recognizes an epitope that is most easily displayed on an α-helix. In the case of group 4 residues, alanine substitution is expected to be far more stabilizing for a helix than either of the native residues (16, 17). Indeed helical stabilizing effects of alanine or alanine substitutions have been observed in both well studied proteins and peptides, such as leucyl-tRNA synthetase (18), T4 lysozyme (19–22), and melittin (23). Locking the antigenic variant peptide into a conformation that most mimics the conformation of the epitope in the original antigen would be expected to slow down the dissociation rate. The finding that only the dissociation rates and not the association rates were affected for group 4 mutants suggests that the antigenic variant peptide epitope for MEM-265 adopts a helix upon complex formation and is largely unstructured in solution. The free peptides are not predicted to be helical in solution (AGADIR predictions). This is consistent with circular dichroism analysis, which indicated that all peptides used in this study exhibited spectra typical of random coil conformations (data not shown). In the context of a protein, however, the sequences of the antigenic variants are expected to have varying degrees of helical character (SSCP predictions).

A helix destabilizing residue, however, in the absence of any immediate stabilizing interactions from its neighboring residues, should increase the dissociation rate. In the case of Glu-52 of group 2, such a scenario may be occurring. Although the substitution of an alanine at Glu-52 is predicted to be more helix stabilizing, the crystal structure of MHC II DR1 reveals that the β-chain residue Glu-52 is within salt bridge distance (<3.0 Å) of Arg-55. The benefit of an Glu-52–Arg-55 salt bridge to helicity may outweigh any positive contribution in this regard offered by E52A. A previously characterized monoclonal antibody raised against tobacco mosaic virus protein was strongly suspected of binding to an epitope displayed on a contiguous face of an α-helix (24). In this particular case, further characterization by alanine scanning and SPR of the antigenic variant peptide corresponding to the original antigen epitope suggested that alanine substitution of a particular glutamic acid, presumably involved in a salt bridge with a vicinal arginine, may affect peptide recognition by stabilizing a conformation that is advantageous to antibody binding (3, 24).

The SpotMatrix SPR fine epitope mapping technique described here provides an excellent means to dissect and understand multiple molecular interactions simultaneously. Assessment of the relative contributions of different residues can be performed at the kinetic level. A more complete and refined understanding of the determinants involved in molecular recognition and specificity can be rapidly and thoroughly derived.
in a manner that minimizes experimental variability by allowing highly parallel measurement of all variants on a single experiment. In addition, a detailed comparative assessment of the association and dissociation rates for a panel of ligands can indicate a structural aspect of molecular recognition exiting between the ligand and analyte. In the present study, an evaluation of the kinetic parameters for the interaction of monoclonal antibody MEM-265 with antigenic variant peptides provided evidence for a model of peptide-antibody recognition reminiscent of an induced fit model (26, 27).

Acknowledgment—We thank Jeremy Lambert for expert technical assistance.

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J. Biol. Chem. 2005, 280:4188-4194.
doi: 10.1074/jbc.M410687200 originally published online November 18, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M410687200

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