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A General and Efficient Approach for NMR Studies of Peptide Dynamics in Class I MHC Peptide Binding Grooves†

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Abstract

T cell receptor recognition of peptides bound by major histocompatibility complex (MHC) proteins initiates a cellular immune response. Dynamics of peptides within MHC binding grooves can influence TCR recognition, yet NMR studies which could address this rigorously have been hindered by the expense of isotopically labeled peptide and the large size of the peptide/MHC complex. Here we describe methodology for characterizing peptide dynamics within MHC binding grooves via NMR, using a biosynthetic approach for producing labeled peptide. With the $Tax_{11–19}$ peptide bound to the human class I MHC HLA-A*0201, we demonstrate that peptide generated in this manner can be well characterized in MHC binding grooves by NMR, providing opportunities to more precisely study the role of peptide dynamics in TCR recognition. Demonstrating the utility of such studies, the data with the $Tax_{11–19}$ peptide indicate the presence of slow conformational exchange in the peptide, supporting an “induced-fit” style TCR binding mechanism.

Recognition of peptides bound and presented by major histocompatibility complex proteins (the pMHC complex[1]) by T cell receptors (TCR) on cytotoxic or helper T cells initiates a signaling cascade leading to a cellular immune response. TCRs on cytotoxic T-cells recognize a composite surface formed by the peptide and the α_1/α_2 helixes of the class I MHC protein. TCR specificity towards different pMHC ligands is usually attributed to conformational differences between the bound peptides (1). Yet emerging data indicate that peptide flexibility within the MHC peptide binding groove can dramatically influence TCR recognition. For example, we recently demonstrated that modifications of the position 2 anchor residue of the Melan-A/MART-$1_{27–35}$ tumor antigen results in enhanced peptide dynamics in the MHC peptide groove and a loss of recognition by antigen-specific T cells (2). As there is growing interest in utilizing the T cell arm of the immune system for the development of vaccines, therapeutic interventions, and imaging reagents, the potential influence of molecular motion in immune recognition, specificity, and cross-reactivity is of considerable significance.

Although crystallography, molecular dynamics simulations, infrared spectroscopy and time-resolved fluorescence anisotropy have been used to examine dynamics of peptides bound to class I MHC molecules (2–4), nuclear magnetic resonance (NMR) relaxation techniques should be expected to provide significant advantages over these techniques due to their ability to resolve residue-specific molecular motions across a range of timescales without the need for

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SUPPORTING INFORMATION AVAILABLE
Expanded methods, R_1 and $R_{1\rho}$ analyses, and MD simulation results. This material is available free at http://pubs.acs.org.

[1]Abbreviations: MHC, major histocompatibility complex; pMHC, peptide/MHC complex; TCR, T cell receptor; CDR, complementarity determining region

an exogenous label. However, NMR studies of peptides in MHC binding grooves have been hindered by the prohibitive cost of isotopically labeled peptides and the large size (~45 kD) of the pMHC complex.

Here, we describe methodology for characterizing the dynamics of peptides in class I MHC peptide binding grooves via NMR using biosynthetic labeled peptide. Although other systems have been described for the biological expression of labeled peptides, a drawback of most is the peptide contains chemically modified or additional amino acids at the N- or C-terminus. Such approaches are suboptimal for producing peptides for incorporation into class I MHC molecules given the length restrictions and requirement for defined N- and C-termini.

To overcome these limitations, we adapted a method in which the peptide of interest was fused to the C-terminus of ubiquitin, in turn modified with an N-terminal hexa-histidine tag (5,6). Ubiquitin is frequently used in NMR methods development and well-developed protocols exist for the expression of large quantities of isotopically labeled protein in bacterial systems (7). For cleavage of the peptide from ubiquitin, we used the ubiquitin hydrolase YUH 1, likewise modified with a C-terminal histidine tag. As YUH 1 cleaves the peptide bond immediately after the terminal glycine of ubiquitin using only the final five ubiquitin amino acids for substrate recognition (8), there are few restrictions on the sequence of the fused peptide, and once cleaved, the peptide is available unmodified. Further, as both the fusion protein and hydrolase possess histidine tags, purification of both proteins and separation of ubiquitin and the hydrolase from the cleaved peptide is straightforward.

As a demonstration system, we used the $Tax_{11–19}$ peptide (Tax; LLFGYPVYV) bound to the class I MHC molecule HLA-A*0201 (HLA-A2). The Tax peptide is bound by HLA-A2 in a usual extended conformation (9), but undergoes a large conformational change when Tax/HLA-A2 is recognized by the A6 and B7 TCRs (10). The change is centered at the backbones of Pro6 and Val7 and, although it is consistent with an induced-fit mechanism, it is possible it is facilitated by conformational selection due to rapid dynamics in the unligated pMHC molecule. NMR dynamics studies of the Tax peptide bound to HLA-A2 can help elucidate the extent to which dynamics contributes to the structurally observed conformational change.

For generating isotopically labeled Tax peptide, the ubiquitin-peptide fusion protein and YUH 1 were expressed separately in *E. coli* BL21(DE3) cells grown in either rich media for YUH 1 or minimal M9 media supplemented with ^{15}N $(NH_4)_2SO_4$ and/or ^{13}C glucose for the ubiquitin-peptide fusion. Three to six hours after induction, cells were harvested. Enrichment of the protein followed commercial protocols. After Ni-NTA affinity column purification, further purification was achieved using size exclusion chromatography. Yields were approximately 40–60 mg of ubiquitin-peptide fusion per liter of cells and 90–120 mg of YUH 1 per liter of cells. β-mercaptoethanol was added to the YUH 1 preparation to a final concentration of 5mM, and the peptide was cleaved using 30% YUH 1 by weight in 500 – 2000 μl reactions at 37 ° for 6 hours. Cleaved peptide was purified from the reaction via a second round of Ni-NTA followed by size exclusion chromatography. Final yields of >99% ^{13}C,^{15}N-labeled peptide were approximately 6–10 mg per liter of cells. Peptide identity and labeling were verified by LC-MS (Fig. 1A).

The method outlined above facilitates rapid and straightforward expression of labeled peptides, yet for performing NMR on pMHC complexes, the complexity of the large protein remains. To help overcome this, the heavy chain of the HLA-A2 complex was deuterated to approximately 98% by bacterial expression of the heavy chain in M9 media (11,12) supplemented with 3g/L ^{2}H Celtone base powder (Fig. 1B). Inclusion body preparation and refolding of the deuterated heavy chain and protonated β_2-microglobulin subunit in the presence of ^{13}C,^{15}N-labeled peptide then followed normal procedure for production of class I

pMHC complexes (13). Coupled with the use of a deuterated, low- conductivity buffer (14), heavy chain deuteration improved the NMR signal by ~ 50%.

Figure 2 shows a two-dimensional ^{1}H-^{15}N-TROSY-HSQC spectrum for Tax bound to HLA-A2 collected at 37 ° in a 18.8 T spectrometer with a cryogenic probe. Insets illustrate the signal enhancement due to heavy chain deuteration as well as exploration of the signal temperature dependence. An experimental temperature of 37 °C was chosen as a trade-off between the increased sensitivity available at higher temperatures and the stability of the pMHC complex. Although the T_m of the Tax/HLA-A2 is near 65 °C (9), other pMHC complexes of lower stability may necessitate lower temperatures for data acquisition.

Assignments for the data shown in Fig. 2 were achieved using HCCH-TOCSY, HCACO, HNCO and HSQC experiments (15–21). Multiple peaks were identified for Val7, possibly reflecting the sampling on a slow timescale of alternate conformations, one of which may correspond to the TCR-bound state in the unligated pMHC complex. The identities of the Val7 peaks were confirmed with a control experiment on Tax/HLA-A2 where the Tax peptide had been prepared via solid-phase synthesis with ^{15}N-labeled valine at position 7.

A saturation transfer experiment was used to examine conformational exchange occurring between the two peaks observed for Val7. When the main peak near 7.5 ppm was irradiated with a saturation time of 2.5 s, no changes were observed in the alternate peak at 7.8 ppm, confirming slow exchange on the NMR timescale.

To demonstrate the capacity to explore peptide dynamics via NMR, we measured ^{15}N longitudinal (R_1) and rotating-frame ($R_{1\rho}$) relaxation rates for all residues of the Tax peptide (excluding Pro6). The resulting rates are summarized in Fig. 3 (accurate relaxation data could only be determined for the predominant Val7 peak). Although there is variation across the peptide, it is interestingly that the rates in the center of the peptide are relatively low, particularly when considering that positions 2 and 9 are primary anchor residues whose side chains are “pinned down” within the peptide binding groove. The centers of antigenic peptides are commonly thought to be the “focus” of the hypervariable CDR3 loops of TCRs (22), and reduced flexibility at the center of the peptide may reflect this. The relatively low rates for Val7 are consistent with the HSQC and saturation transfer experiments indicating slow conformational exchange, and suggest that any sampling of the TCR-bound conformation in the unligated pMHC likewise occurs on a slow timescale.

To follow up on the dynamical behavior for Val7, we examined the flexibility of the Tax peptide bound to HLA-A2 by molecular dynamics (MD) simulations, using an unrestrained MD protocol we recently employed with the MART-1/Melan-A tumor antigen (2). Over the course of a 30 ns simulation at 300K, the backbone of Val7 populated two distinct conformations, with the crystallographically observed conformation populated 85% of the time and an alternative conformation that has not been observed in any structure populated 15% of the time (Supporting Fig. S3A). Increasing the simulation temperature to 330K dropped the percentage of the unligated conformation to 81%. Notably though, at the elevated temperature the TCR-bound conformation was populated only 2% of the time (Supporting Fig. S3B). These observations are consistent with two predictions from the NMR data. First is the existence of multiple peptide conformations separated by high barriers, particularly for Val7. Second is that the conformational change around Pro6/Val7 that occurs upon binding of the A6 and B7 TCRs may reflect more of an “induced-fit” style binding mechanism rather than a mechanism based on conformational selection in the unbound pMHC.

In summary, we have demonstrated a methodology for collecting high resolution NMR data on peptides bound within class I MHC peptide binding grooves. The approach is entirely general and can be easily adapted to different peptides and MHC molecules. As demonstrated

with the Tax peptide, application of this approach can be expected to help reveal the influence peptide molecular motion plays in T cell recognition of antigen. Given the growing appreciation for the role of molecular flexibility in influencing TCR recognition of pMHC (23), as well as the expanding interest in the development of peptide/MHC-based vaccines, therapeutic agents, and imaging tools, NMR characterizations of peptide dynamics should have significant impact in both basic and applied immunology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Madden DR. Annual Review of Immunology 1995;13:587–622.
2. Borbulevych OY, Insaidoo FK, Baxter TK, Powell DJ Jr, Johnson LA, Restifo NP, Baker BM. J Mol Biol 2007;372:1123–1136. [PubMed: 17719062]
3. Pohlmann T, Bockmann RA, Grubmuller H, Uchanska-Ziegler B, Ziegler A, Alexiev U. J Biol Chem 2004;279:28197–28201. [PubMed: 15084610]
4. Fabian H, Huser H, Narzi D, Misselwitz R, Loll B, Ziegler A, Bockmann RA, Uchanska-Ziegler B, Naumann D. J Mol Biol 2008;376:798–810. [PubMed: 18178223]
5. Kohno T, Kusunoki H, Sato K, Wakamatsu K. J Biomol NMR 1998;12:109–121. [PubMed: 9729791]
6. Mildner AM, Paddock DJ, LeCureux LW, Leone JW, Anderson DC, Tomasselli AG, Heinrikson RL. Protein Expr Purif 1999;16:347–354. [PubMed: 10419831]
7. Fiaux J, Bertelsen EB, Horwich AL, Wuthrich K. J Biomol NMR 2004;29:289–297. [PubMed: 15213427]
8. Johnston SC, Riddle SM, Cohen RE, Hill CP. EMBO J 1999;18:3877–3887. [PubMed: 10406793]
9. Khan AR, Baker BM, Ghosh P, Biddison WE, Wiley DC. J Immunol 2000;164:6398–6405. [PubMed: 10843695]
10. Ding YH, Smith KJ, Garboczi DN, Utz U, Biddison WE, Wiley DC. Immunity 1998;8:403–411. [PubMed: 9586631]
11. Marley J, Lu M, Bracken C. J Biomol NMR 2001;20:71–75. [PubMed: 11430757]
12. Cai ML, Huang Y, Sakaguchi K, Clore GM, Gronenborn AM, Craigie R. J Biomol NMR 1998;11:97–102. [PubMed: 9566315]
13. Garboczi DN, Hung DT, Wiley DC. Proc Natl Acad Sci U S A 1992;89:3429–3433. [PubMed: 1565634]
14. Kelly AE, Ou HD, Withers R, Dotsch V. J Am Chem Soc 2002;124:12013–12019. [PubMed: 12358548]
15. Kay LE, Ikura M, Tschudin R, Bax A. J Magn Reson 1990;89:496–514.
16. Kay LE, Keifer P, Saarinen T. J Am Chem Soc 1992;114:10663–10665.
17. Kay LE, Xu GY, Singer AU, Muhandiram DR, Formankay JD. J Mag Res B 1993;101:333–337.
18. Bodenhausen G, Ruben DJ. Chem Phys Letters 1980;69:185–189.
19. Piotto M, Saudek V, Sklenar V. J Biomol NMR 1992;2:661–665. [PubMed: 1490109]
20. Sklenar V, Piotto M, Leppik R, Saudek V. J Mag Res A 1993;102:241–245.
21. Salzmann M, Pervushin K, Wider G, Senn H, Wuthrich K. Proc Natl Acad Sci USA 1998;95:13585–13590. [PubMed: 9811843]
22. Garboczi DN, Biddison WE. Immunity 1999;10:1–7. [PubMed: 10023765]
23. Armstrong KM, Piepenbrink KH, Baker BM. Biochem J 2008;415:183–196. [PubMed: 18800968]

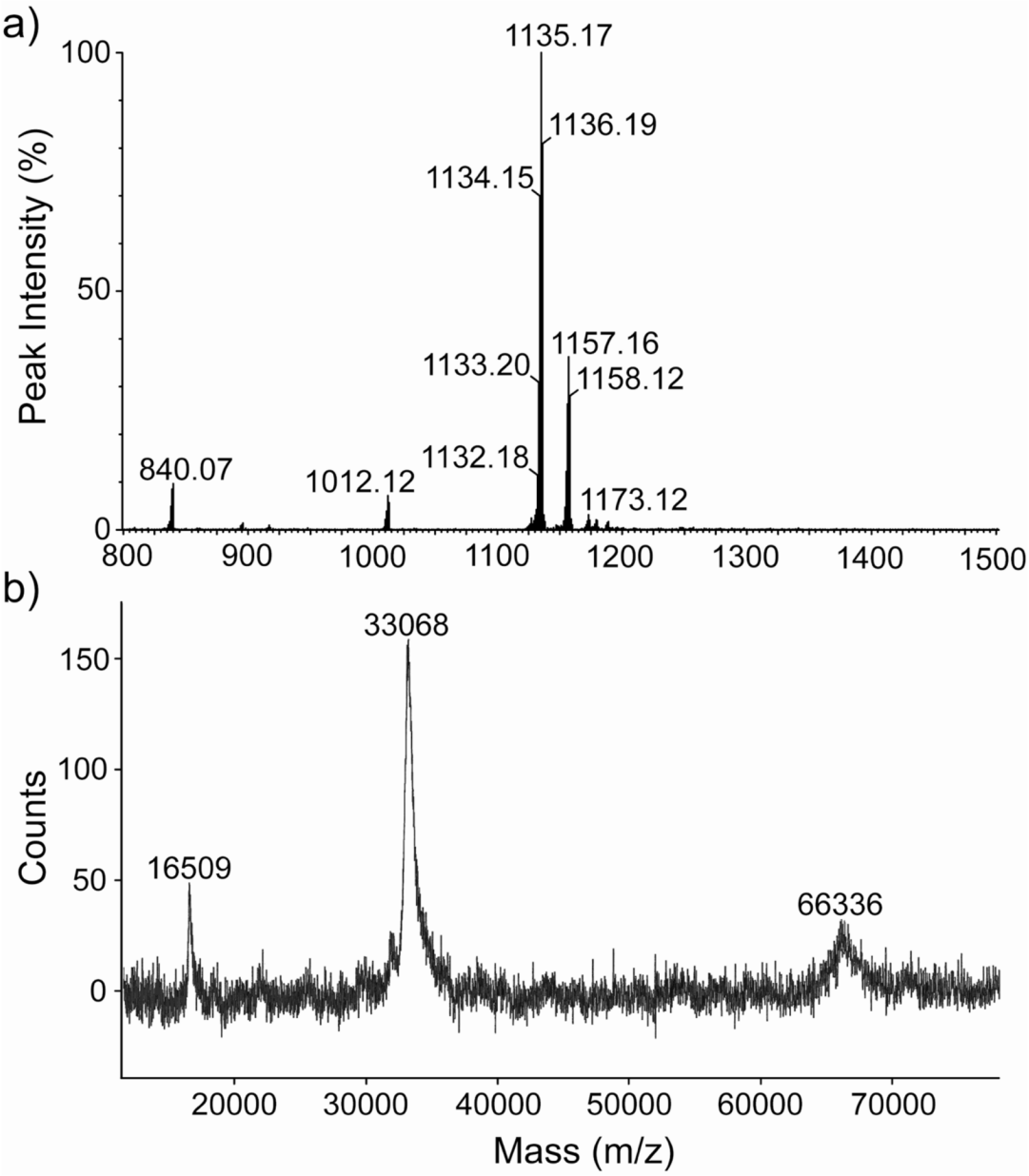

FIGURE 1.

Isotopic labeling of the Tax peptide and the HLA-A2 heavy chain. A) Mass spectral analysis of the purified biosynthetic [^{13}C,^{15}N]-labeled Tax peptide indicates near complete labeling. The calculated molecular weight of uniformly labeled Tax peptide is 1135 Daltons, whereas unlabeled peptide is 1070 (the peak at 1157 represents a sodium adduct). B) MALDI-TOF mass spectral analysis of deuterated HLA-A2 heavy chain shows a strong peak at 33,068 Daltons. The molecular weight of unlabeled heavy chain is 31,930 Daltons, indicating approximately 98% deuteration.

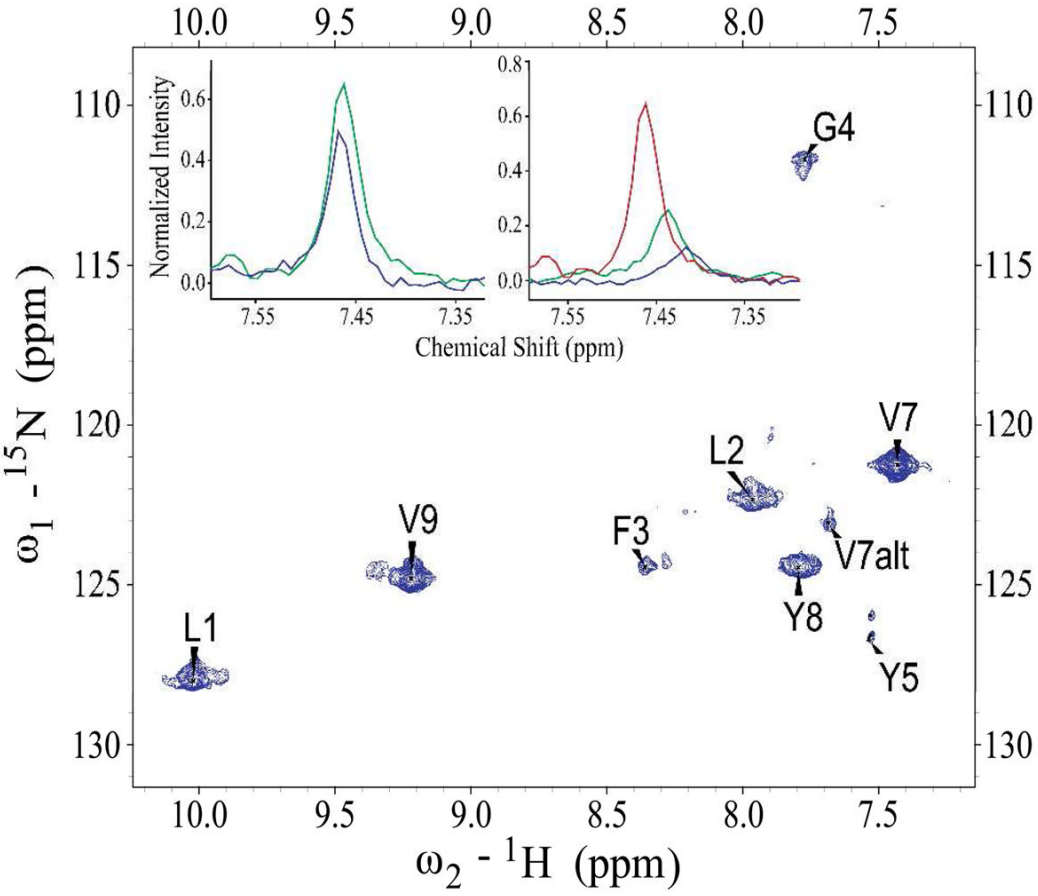

FIGURE 2.

Two dimensional ^{1}H-^{15}N TROSY-HSQC of [^{13}C,^{15}N]-Tax bound to HLA-A2. Data were acquired on a Bruker Avance 800 MHz (18.8 T) spectrometer in 20 mM ^{2}H-bis-tris with ^{2}H benzoic acid, pH 7.4, 90 % H_2O and 10% $^{2}H_20$ at 37 °C. V7alt indicates a weak, alternate peak for Val7. The left inset indicates signal enhancement for the major Val7 peak from deuteration of the HLA-A2 heavy chain and use of a deuterated, low conductivity buffer. The right inset shows the temperature dependence of the signal strength for the major Val7 peak (25 °C in blue; 35 °C in green; 45 °C in red).

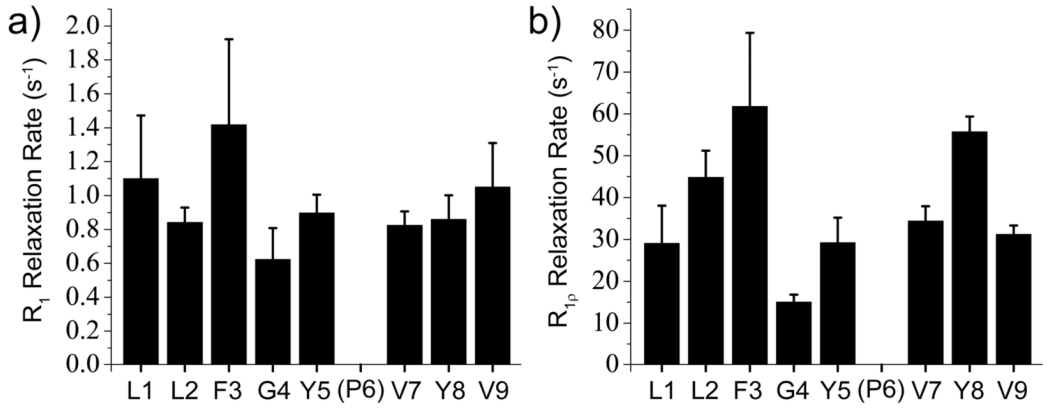

FIGURE 3.

Longitudinal (A) and rotating-frame (B) relaxation rates for [^{13}C,^{15}N]-Tax peptide bound to HLA-A2 reveal differential dynamics across the bound peptide.