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Disparate Degrees of Hypervariable Loop Flexibility Control T-Cell Receptor Cross-Reactivity, Specificity, and Binding Mechanism

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αβ T-cell receptors (TCRs) recognize multiple antigenic peptides bound and presented by major histocompatibility complex molecules. TCR cross-reactivity has been attributed in part to the flexibility of TCR complementarity-determining region (CDR) loops, yet there have been limited direct studies of loop dynamics to determine the extent of its role. Here we studied the flexibility of the binding loops of the αβ TCR A6 using crystallographic, spectroscopic, and computational methods. A significant role for flexibility in binding and cross-reactivity was indicated only for the CDR3α and CDR3β hypervariable loops. Examination of the energy landscapes of these two loops indicated that CDR3β possesses a broad, smooth energy landscape, leading to rapid sampling in the free TCR of a range of conformations compatible with different ligands. The landscape for CDR3α is more rugged, resulting in more limited conformational sampling that leads to specificity for a reduced set of peptides as well as the major histocompatibility complex protein. In addition to informing on the mechanisms of cross-reactivity and specificity, the energy landscapes of the two loops indicate a complex mechanism for TCR binding, incorporating elements of both conformational selection and induced fit in a manner that blends features of popular models for TCR recognition.

Introduction

Elimination of pathogens by the T-cell arm of the immune system requires T-cell recognition of an antigenic peptide bound and presented by class I or class II major histocompatibility complex (MHC) proteins. Recognition occurs via the T-cell receptor (TCR), a clonotypic, heterodimeric cell surface receptor. A defining characteristic of TCRs is their capacity to recognize multiple peptide/MHC (pMHC) ligands, necessary due to the fixed size of the T-cell repertoire relative to the larger array of potential antigens.[1] Apart from ensuring reactivity against antigens derived from pathogens, TCR cross-reactivity is also necessary in the development and maintenance of the T-cell repertoire and is

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Abbreviations used: TCR, T-cell receptor; CDR, complementarity-determining region; MHC, major histocompatibility complex; pMHC, peptide/MHC; HLA-A2, HLA-A*0201; MD, molecular dynamics; TRFA, time-resolved fluorescence anisotropy; TCSPC, time-correlated single-photon counting; ITC, isothermal titration calorimetry; PDB, Protein Data Bank; NIGMS, National Institute of General Medical Sciences.

believed to underlie various autoimmune pathologies and the rejection of transplanted tissues. Yet TCRs are not highly degenerate, showing specificity for particular peptide subsets and (via the phenomenon of MHC restriction) usually recognizing peptides presented by a subset of MHC alleles.

Flexibility of the TCR antigen binding site is frequently discussed as an underlying contributor to cross-reactivity. Binding site flexibility has been inferred from the crystallographic structures of bound and free TCRs, which frequently show differences in the conformation of complementarity-determining region (CDR) loops (reviewed by Armstrong *et al.*[2]). Flexibility has also been inferred from multiple structures of the same TCR bound to different pMHC ligands, in which CDR loops often adopt different conformations (e.g., Jones *et al.*,[3] Mazza *et al.*,[4] and Reiser *et al.*[5]). Significant attention has been paid to the hypervariable CDR3 loops, which usually form the most interactions with the peptide in TCR–pMHC crystal structures.[6] Moreover, when bound and free TCRs are compared, the CDR3 loops show the largest overall changes in conformation.[2]

Conformational changes upon binding, such as those seen for TCR CDR3 loops, are often attributed to induced-fit-type motions occurring after initial contact. Induced fit is embodied in the two-step mechanism for TCR cross-reactivity, which proposes that the TCR adjusts to the peptide after initial contact with MHC.[7] An alternative (but not mutually exclusive) mechanism for conformational changes upon binding is the "selection" of a compatible conformation from a preexisting structural equilibrium.[8,9] For TCRs, conformational selection is embodied in the "conformer" model, which proposes that distinct conformations of a TCR generated via a preexisting equilibrium maintain specificity for different ligands.[10]

In recent years, both conformational selection and induced fit have received considerable attention as general mechanisms for protein binding and selectivity.[8,9,11] Both reflect the underlying structural and energetic landscapes of interacting molecules, with the actual binding mechanism dependent on the "roughness" of these landscapes (i.e., the energies of various conformational substates and the height of the barriers between them). Yet while both induced fit and conformational selection have been postulated to play roles in TCR binding and cross-reactivity, there have been few studies evaluating the intrinsic flexibilities of TCR binding loops. An NMR study of the D10 TCR reported greater flexibility of the CDR3α and CDR3β loops on the picosecond timescale.[12] Stopped-flow kinetic measurements have shown that the interaction of a Cytomegalovirus peptide-specific TCR with its ligand is rate-limited by an induced-fit mechanism, but the location and magnitude of the associated structural changes that occur during binding are unknown.[13] Thermodynamic studies have suggested that a number of TCRs must undergo conformational changes during binding (reviewed by Armstrong *et al.*[14]), but these are unable to address specific changes and cannot discriminate between binding mechanisms. The resulting uncertainty about the intrinsic flexibility of CDR3 loops has led to characterizations ranging from unstructured loops that require folding upon binding[7] to ordered loops that undergo remodeling or rigid-body shifts upon binding.[15] That different CDR3 loop sequences will invariably possess different degrees of flexibility adds further complications.

The αβ TCR A6 is among the most well-characterized TCRs, with crystallographic structures available for the TCR bound to nine ligands. These include the Tax peptide (LLFGYPVYV),[16] the HuD peptide (LGYGFVNYI),[17] the Tel1p peptide (MLWGYLQYV),[18] and six single amino acid variants of the Tax peptide,[19–21] all presented by the class I MHC HLA-A∗0201 (HLA-A2). These structures comprise the largest structural database available for a single TCR. A distinctive feature of this database is the variability in the conformation of the CDR3β loop, which adjusts significantly in response to different ligands. In contrast, the positions of CDR3α and the remaining loops are independent of the peptide. This structural database, together with available data on A6 binding, specificity, and cross-reactivity, provides a unique opportunity for studying the structural and energetic landscapes of a TCR's CDR loops and for establishing their roles in binding and cross-reactivity.

We began by determining the structure of the free A6 TCR, which revealed that both the CDR3α and the CDR3β hypervariable loops must undergo conformational adjustments in order to bind. However, in the free TCR, the CDR3α and CDR3β loops possess different degrees of flexibility, as shown by time-resolved fluorescence measurements. Together with molecular dynamics (MD) simulations and additional structural and thermodynamic data, our results indicate that CDR3β possesses a relatively smooth, broad energy landscape, allowing the free TCR to rapidly sample a range of conformations that are compatible with ligands possessing structural and chemical heterogeneity across the center of the peptide. The landscape of CDR3α is more rugged, leading to slower and more restrained motion that restricts the receptor to a more defined set of peptides and likely only those presented by HLA-A2. Altogether, the data indicate that cross-reactivity and specificity are preprogrammed into the energy landscapes of the A6 TCR's hypervariable loops, with the TCR interacting via a mechanism that blends elements of both conformational selection and induced fit and