The Crystal Structure of a Pyrrolinone−Peptide Hybrid Ligand Bound to the Human Class II MHC Protein HLA-DR1

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Abstract: The X-ray crystal structure of a complex between the human class II major histocompatibility complex (MHC) protein HLA-DR1 and a bispyrrolinone−peptide hybrid ligand has been determined to 2.7 Å resolution. The bispyrrolinone segment of the ligand closely mimics the polyproline type II conformation of peptide ligands bound to class II MHC molecules, emphasizing the considerable versatility of this peptidomimetic scaffold. Most hydrogen bonds conserved in all peptide/class II complexes are formed, and the side chains of the bispyrrolinone segment project into the same spaces as those occupied by side chains of bound peptides. Molecular modeling used in the design of the hybrid ligand was remarkably accurate in predicting the observed molecular interactions.

Introduction

Class II MHC molecules, which are found on specialized antigen-presenting cells, bind antigenic peptides derived from extracellular and intravesicular antigens for recognition by the T cell receptors of $CD4^+$ T lymphocytes to regulate the immune response.[1,2] A number of different alleles of class II molecules are found in the human population. Specific alleles have been linked to increased susceptibility to particular autoimmune disorders: HLA-DR2 to multiple sclerosis, HLA-DQ8 to insulin-dependent diabetes mellitus, and HLA-DR1 and HLA-DR4 to rheumatoid arthritis.[3] Inhibition of $CD4^+$ T cell recognition of self-antigens presented by specific class II MHC alleles holds the promise of therapeutic intervention in autoimmune diseases.[4]

Crystallographic studies established that antigenic peptides bind to class II MHC molecules in an extended, polyproline type II conformation with the peptide backbone twisted such that side chains project off every 120°.[5−7] Approximately 12 of the main chain polar groups of bound peptides form hydrogen bonds to conserved atoms found on all class II MHC alleles. It has been proposed that the common feature of the conserved peptide-to-MHC hydrogen bonds could provide the basis for the extremely long bound half-lives of peptides, often measured to be hundreds of hours.[8]

On the basis of the X-ray structure of HLA-DR1 complexed with the influenza hemagglutinin peptide HA 306-318[5] and peptide analogue data originating with M13 phage nonapeptide libraries,[9] the Roche group did a systematic study of peptide mimetic inhibitors of MHC class II binding finding that extensive truncation and backbone replacement was feasible without compromising binding affinity.[10] Mimetic substitutions that blocked cathepsin B cleavage generated potent inhibitors of protein antigen presentation and T-cell proliferation. These results suggested that the mimetics were able to survive and compete with protein-derived antigenic peptide fragments in the endosomal compartment where the MHC molecules are loaded. Other studies have been reported in which the HLA-DR1 structure was used to design inhibitors of in vitro peptide binding

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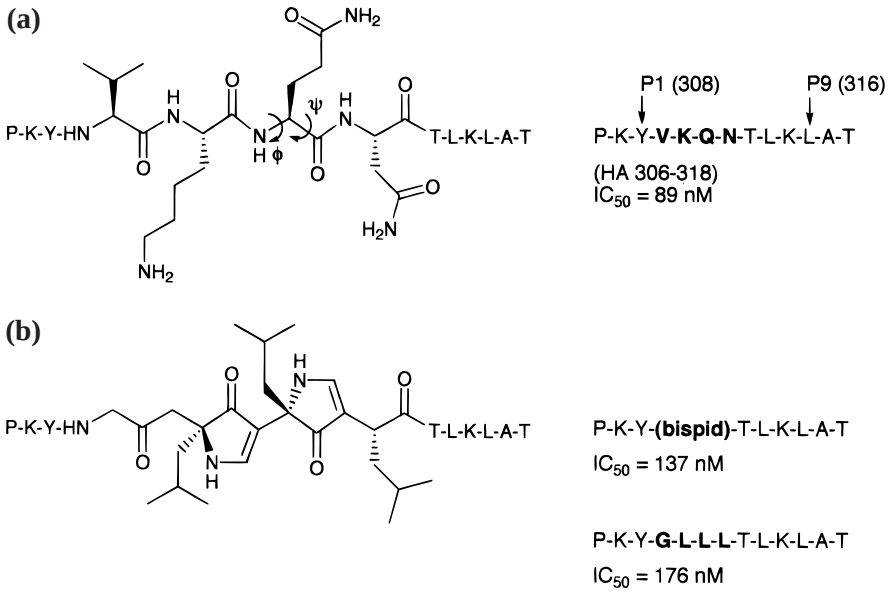

Figure 1. Chemical structure of the pyrrolinone−peptide hybrid ligand and the HA peptide. (a) HA peptide shown in single letter amino acid code. Peptide positions labeled above peptide follow numbering in ref 5, The four residues replaced in the hybrid ligand are shown in bold. (b) Bispyrrolinone−peptide hybrid ligand with peptide residues shown in single letter code. IC_{50} values of the HA peptide (a) and the competitive inhibitors (b) are shown. Figure adapted from Smith et al.[23] and generated with ISIS/Draw.

to HLA-DR1 that resemble tetrapeptides[11−14] or longer peptidic ligands.[15] Inhibitors of MHC class I molecules have also been reported.[16−20]

At the University of Pennsylvania, pyrrolinones were designed as mimetics of β-turns, β-strands, and β-pleated sheets[21] by replacing amide bonds with a scaffold that permitted interstrand hydrogen bonding. Peptidomimetics based on a 3,5,5 pyrrolin-4-one scaffold have many properties of peptide chains. They contain carbonyl groups spaced every three atoms along a main chain like peptides. The backbone is, however, all carbon atoms with nitrogen present, but displaced from the backbone and cyclized to generate pyrrolinone rings (Figure 1a). The ring provides rigidity, constraining the ψ and ω angles (defined in Figure 1a). Importantly, pyrrolinones can incorporate amino acid side chains that have trajectories such as peptides. Because of the absence of a peptide bond pyrrolinones are resistant to proteases. Crystallography and solution NMR indicate that the pyrrolinone scaffold can mimic extended β-strands and some helical secondary structures.[22−24] Improved membrane transport properties, relative to peptides, have been ascribed to the ability of bispyrrolinones to form an intramolecular hydrogen bond between the amine and carbonyl groups of successive pyrrolinone rings.[24] Potent inhibitors of the aspartyl proteases renin and HIV-1 protease have been reported based on mono- and bispyrrolinones.[24−28]

We report here the X-ray structure determination of an HLA-DR1 bound inhibitor, PKY(bispyr)TLKLAT (IC_{50} 137nM), that incorporates a bispyrrolinone having a glycine-like linker at P2 and three leucine-related side chains at P3−5 (Figure 1).[23] The bispyrrolinone−peptide hybrid has an essentially equivalent binding affinity to the native HA peptide (IC_{50} 89 nM) and to the reference HA analogue having the GLLL sequence substituted for VKQN (Figure 1).[23] The bispyrrolinone−peptide hybrid binds almost exactly as modeled during the design of the inhibitor.[28] The side chains of the tetrapeptide-mimic project into the same spaces as the peptide side chains they replace, and the bispyrrolinone backbone makes all but one of the

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Table 1. Crystallographic Statistics

Data Statistics	
number of independent reflections	33499
resolution range	45−2.6 Å
completeness	95.2%
R merge	7.3% (39%)[a]
space group	$C222_1$
cell parameters	$a = 96.51$ Å $b = 111.75$ Å $c = 206.06$ Å
no. of molecules in au	2
Refinement Statistics	
resolution range	35.0−2.7 Å
no of reflections ($F > 2s$)	33067
no. of atoms	6370
R_{free}[b]	26.65% (0.35%)
R_{work}[b]	22.46% (0.32%)
rms deviations	
bond length	0.0076 Å
bond angle	1.40°

[a] Statistics in 2.6−2.7 Å shell. [b] Statistics in 2.7−2.73 Å shell. [b] $R_{free} = (\Sigma_h |F_o - F_c|)/(\Sigma_h F_o)$, $\forall h \in \{\text{free set}\}$; $R_{work} = (\Sigma_h |F_o - F_c|)/(\Sigma_h F_o)$, $\forall h \in \{\text{working set}\}$. $R_{merge} = (\Sigma_{hkl} |I - \langle I \rangle|)/(\Sigma_{hkl} \langle I \rangle)$, $\forall hkl \in \{\text{independent Miller indices}\}$.

conserved hydrogen bonds with the HLA molecule. The bispyrrolinone backbone adopts a polyproline type II-like conformation, like bound peptides, emphasizing the versatility of this mimetic scaffold.

Results

Structure Determination. The soluble ectodomain of ligand-free HLA-DR1 (empty DR1) expressed in insect cells was loaded with the bispyrrolinone−peptide hybrid ligand by incubation in excess ligand for 72 h. The resulting DR1/ligand complex was purified by gel filtration chromatography and crystallized (see methods). X-ray data were collected to 2.6 Å resolution and the structure determined by molecular replacement. The refined structure ($R_{work} = 0.22$; $R_{free} = 0.27$) clearly showed the entire bound ligand (X-ray statistics, see Table 1).

Interactions of Bispyrrolinone−Peptide Hybrid Ligand with HLA-DR1. The peptide segments of the hybrid ligand that are shared with the HA peptide, residues P-2 to P1 (PKY) and P6 to P11 (TLKLAT), are bound to DR1 essentially indistinguishably from the way those segments of the HA peptide bind (compare Figure 2a to Figure 3a of ref 5). The same array of hydrogen bonds (dashed lines in Figure 2a) are formed from main chain atoms of the hybrid ligand to conserved residues of the class II MHC molecule (underlined in Figure 2a) and the same side chains fit into pockets in the DR1 binding cleft (see Figure 4 of ref 5).

The bispyrrolinone containing-segment of the hybrid ligand (red solid lines in Figure 2a), where the HA peptide side chains of P2 to P5 (VKQN) are replaced by the side chains of GLLL on a bispyrrolinone scaffold, also binds very similarly to the HA peptide. The electron density at 2.6 Å resolution is sufficiently detailed to allow the carbonyl oxygens of the bispyrrolinone to be placed, permitting the hydrogen bond network to be modeled (Figure 2b). Only one of the hydrogen bonds from the main chain of the ligand to conserved residues on the class II molecule is missing, that from Asn-β82 to the main chain carbonyl oxygen of P2 Gly (red dashed line in Figure 2a). The leucine-like side chains at positions 3, 4, and 5 of the hybrid ligand project into the same positions as the side chains of Lys-310, Gln-311, and Asn-312 of the HA peptide (positions labeled 3, 4, 5 in Figure 2c and 2d), mimicking the polyproline type II conformation of bound peptides.[6,7]

Bispyrrolinone Binding Compared to HA Peptide Binding. The bispyrrolinone scaffold successfully mimics the peptide main chain interactions with DR1. The carbonyl oxygen of the second pyrrolinone ring (position 4 Figure 2a, c) makes the same hydrogen bond to the conserved DR1 residue Asn-α62 as the carbonyl oxygen of P4 (Gln-311) of the HA peptide. That pyrrolinone carbonyl oxygen is, however, not within hydrogen bonding distance of the nonconserved DR1 residue Gln-α9 (red dashes in Figure 2a; compare Figure 2c and d). The hydrogen bond from the side chain oxygen of DR1 Gln-α9 to the amide of the P4 position of the HA peptide is mimicked by a hydrogen bond from the same DR1 atom to the "displaced amide" of the first pyrrolinone ring at position 3 (Figure 2a, c, d).

Two hydrogen bonds from DR1 to ligand side chains are lost as a result of the substitution of nonpolar leucine side chains in the hybrid ligand for P3 Lys-311 and P5 Asn-313 of the HA peptide: the hydrogen bonds from the ϵ-amino group of Lys-310 to DR1 Asn-α62 and from the ϵ-amide group of Gln-311 to DR1 Gln-β70 (red dashes in Figure 2a and 2d).

There are a number of small differences in the structure of DR1 in the DR1/hybrid-ligand complex when compared to the DR1/HA complex. The distal atoms of DR1 Gln-β70, for example, are relocated by approximately 3.5 Å permitting the leucine-like side chain of the second pyrrolinone to occupy the P4 pocket in place of Gln-311 of the HA peptide, which had formed a hydrogen bond with DR1 Gln-β70. Minor differences of about 1 Å or less are also found in the positions below the hybrid-ligand, such as at DR1 positions α9, α24, and β13, presumably to accommodate the differences in the ligand structures.

The dihedral angles, ϕ and ψ, of amino acids in peptides bound to class II molecules cluster around those of the extended polyproline type II conformation, $\phi = \sim -78°$ and $\psi = \sim 145°$. Table 2 shows that the corresponding dihedral angles in the bispyrrolinone segment of the ligand also maintain a pseudo-polyproline type II conformation (dihedral angles defined in Figure 1). This emphasizes the ability of the bispyrrolinone scaffold to adopt a number of the common conformations of polypeptides.

Although the positions and contacts of both the main chain and side chain of the hybrid ligand mimic the HA peptide very well, the bispyrrolinone segment of the hybrid ligand is positioned not quite as deep into the binding site as the corresponding segment of the HA peptide (Figure 3a). Comparing the α carbon positions of the HA peptide to the carbons of the hybrid ligand from which the amino acid-like side chains emerge, the positions match almost perfectly at P2 and P5 (<0.5 Å) but are displaced by 1.08 and 0.99 Å at P3 and P4, respectively.

The solvent accessible surface area of the bispyrrolinone segment of the peptide hybrid ligand that becomes buried upon binding to DR1 (red in Figure 2a) is 262 Å^2, almost exactly the same as corresponding region of the HA peptide, 264 Å. In both cases about 120 Å^2 of the solvent accessible surface of these segments of the ligands remains exposed and could be contacted by T cell receptors.

Bispyrrolinone Ligand Binding Compared to Molecular Modeling Used in Its Design. Molecular modeling of the interaction between the bispyrrolinone ligand and DR1 predicted the binding mode observed in the X-ray structure remarkably well[23] (Figure 3b). The overall positioning and orientation of both the main chain and side chain atoms were correct in the model. The hydrogen bond from the second pyrrolinone ring carbonyl to DR1 Asn-α62 was predicted correctly. Another

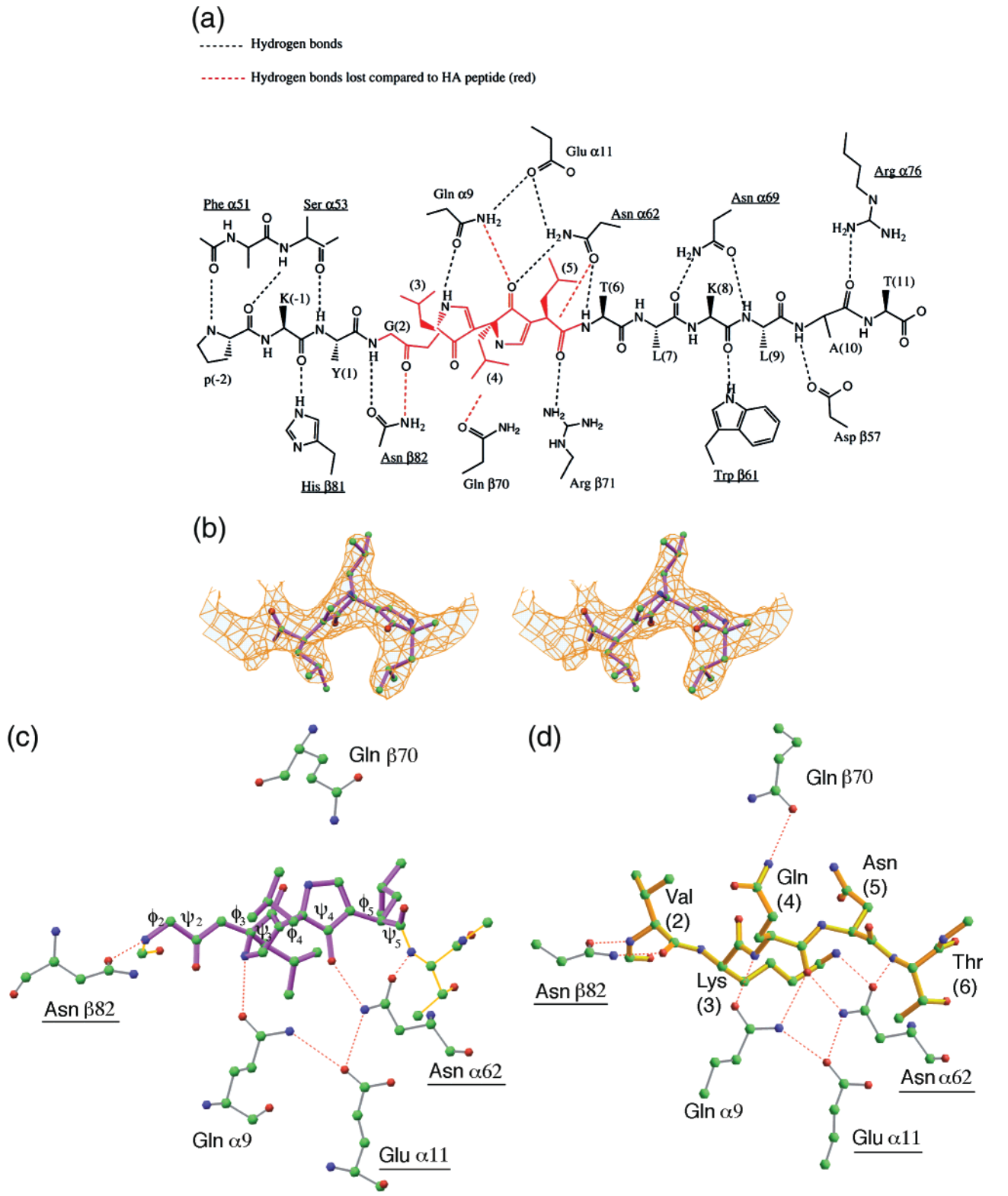

Figure 2. HLA-DR1 interactions of the pyrrolinone−peptide hybrid ligand and the HA peptide. (a) Hydrogen bonds from the bispyrrolinone−peptide hybrid ligand to HLA-DR1 (black dashes). Conserved DR residues are underlined. Hydrogen bonds present from HA peptide to DR1, missing in hybrid ligand/DR1 complex (red dashes). Segment replaced in the bispyrrolinone ligand is colored red. (b) Stereographic diagram of the bispyrrolinone electron density. A simulated-annealing "omit" electron density map ($2F_o - F_c$) contoured at 1σ[37] of the bispyrrolinone segment of the hybrid ligand showing the locations of the carbonyl groups that permits modeling the hydrogen bonding network in (c). (c) Pyrrolinone−peptide hybrid ligand (shown from P2 to P6) including probable hydrogen bonds (red dashes) to DR1 (gray) and dihedral angles. (P2−P5 = purple; flanking peptide = yellow; atom colors: carbon = green; nitrogen = blue; oxygen = red). (c) HA (306−318) peptide (yellow) including probable hydrogen bonds (red dashes) to DR1 (gray). (Atom colors: carbon = green; nitrogen = blue; oxygen = red). Figure generated with Ribbons.[38]

hydrogen bond predicted to be from the same ligand carbonyl group to DR1 Gln-α9 was not observed but is apparently replaced by a hydrogen bond from the amide of the first pyrrolinone ring to DR1 Gln-α9 (Figure 2c).

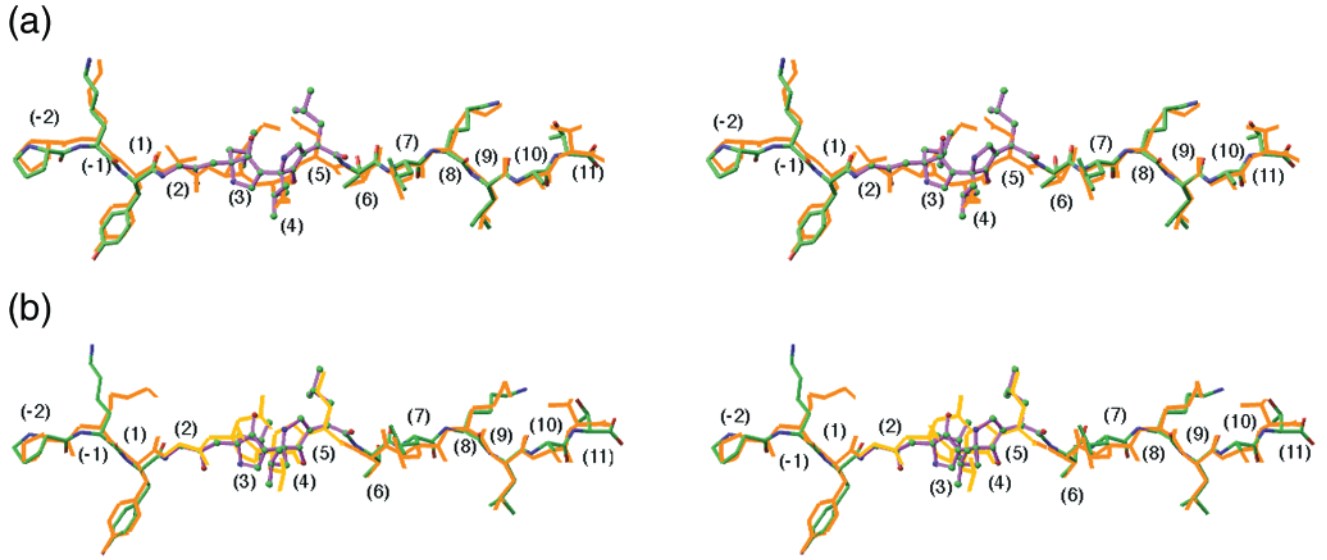

Figure 3. (a) Stereo diagram comparing the DR1 bound conformations of the pyrrolinone−peptide hybrid ligand (purple and green) and the HA peptide (thin orange lines). The ligand is oriented such that the MHC molecule would be at the bottom of the figure and a T cell receptor would approach from the top. Peptide positions bound to HLA-DR1 are in parentheses. (b) Stereo diagram comparing the DR1 bound conformations of the pyrrolinone−peptide hybrid ligand (purple and green) and a model of the bound ligand used in its design (thin orange lines).[23] Figure generated with Ribbons.[38]

Table 2. Dihedral Angles of the Bound Ligand, the HA Peptide, and a Molecular Model

	bound ligand		molecular model		HA peptide	
position	ϕ	ψ	ϕ	ψ	ϕ	ψ
2	−79.5°	154.0°	−94.7°	100.1°	−95.9°	117.6°
3	−127.6°	125.7°	−43.5°	134.0°	−87.4°	165.4°
4	−100.0°	111.0°	−126.6°	115.4°	−122.5°	136.6°
5	−60.8°	124.6°	−69.1°	132.8°	−81.6°	149.8°

Most of the dihedral angles of the modeled bound ligand, listed in Table 2 are near those observed, with two notable exceptions. ψ of position 2 and ϕ of position 3 both differ by more than 50°, from the molecular modeling result. These differences are partially responsible for the difference between the modeled and the observed location of the first pyrrolinone ring (Figure 3b, c), which modeling suggested would position the pyrrolinone NH group above a nonpolar cavity containing three phenylalanines α22, α24, and α54. A second-generation ligand was designed to incorporate an *N*-methyl substitution on this amide to prevent the polar NH from being forced into this cavity.[23] That ligand also included an isopropyl group to mimic valine as in the HA peptide in place of the glycine-like linker at P2. The observed structure suggests that the valine-like side chain could have been accommodated, but that the pyrrolinone ring is positioned to form a hydrogen bond from its amide group with DR1 Gln-α9, leaving no space for an *N*-methyl substituent. This may explain the 10-fold reduction in affinity (IC_{50} = 1.39 μM) of this ligand relative to the one studied here.[23]

Discussion

The molecular modeling used to design the bispyrrolinone−peptide hybrid ligand of HLA-DR1 based on the structure of a peptide/DR1 complex[5,28] is strongly validated by the very close correspondence between the design-model and the X-ray structure of the protein bound inhibitor (Figure 3b). The versatility of the pyrrolinone scaffold, earlier established to mimic β-sheet, β-turn, and helical conformations,[22,23] is extended by the observation that it mimics the polyproline type II conformation of peptides bound to class II MHC molecules. Both the carbonyl groups and the side chains of the bound inhibitor are positioned like the corresponding elements of an antigenic peptide with the formation of main chain-to-protein hydrogen bonds and the interaction of side chains with specific pockets of the DR1 peptide binding site, as observed earlier for peptides bound to class II MHC molecules.

The observation that the P4 side chain of the pyrrolinone segment projects into the P4 pocket of DR1, suggests that placing specific side chains at that position on mimetics could help provide allelic binding specificity to HLA-DR2 and HLA-DR4, where those positions are important for specific binding and have also been implicated in the specificity for different autoimmune disorders.[6,29,30] The bispyrrolinone, however, has low affinity for HLA-DR4, while the reference peptide with GLLL replacing VKQN is approximately equipotent on the two alleles. Thus, allele specificity may be more complex than simple side chain adaptation. On the basis of the successful mimicry of the polyproline helix by the pyrrolinone mimetics, a further extension of the pyrrolinone system toward the critical P1 pocket[31] by adding a second bispyrrolinone unit would seem feasible. Such a compound, however, might not make all of the backbone hydrogen bonds to Asn β82. Extensions in the direction of P7 might be more likely to succeed since less contact with the backbone is seen to that point. The close correspondence between the amount of solvent-accessible surface buried on the bispyrrolinone and the antigenic peptide segment it mimicked is consistent with the comparable affinities of the hybrid inhibitor and the antigenic peptide. This correspondence in buried surface area and the ability of the bispyrrolinone−peptide hybrid to mimic the polyproline type II secondary structure of class II bound peptides suggests that a **poly**pyrrolinone mimic could have the potential to encompass the entire binding site. These types of variants could lead to compounds that lack standard amide bonds, yet are capable of interacting with MHC class II binding sites. They may also

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represent an alternate approach to non-immunogenic inhibitors by having a shortened length[32] or by including only small side chains that do not extend out of the binding site where they can be sensed by the TCR. The pyrrolinone system hypothetically could be adapted to produce inhibitors that block TCR recognition by having bulky appendages to interfere with TCR contact.

Methods

Protein Purification and Crystallization. Ligand-free HLA-DR1 (empty DR1) was expressed in Schneider cells and purified by an immuno-affinity column conjugated with anti-DR monoclonal antibody LB3.1 as described previously.[6] The HA/bispyrrolinone hybrid ligand was loaded onto empty DR1 by incubation of a 5-fold molar excess of ligand with empty DR1 at 37 °C for 72 h. The DR1/HA−bispyrrolinone complex was purified by gel filtration chromatography using a Superdex 200 column in PBS and concentrated to 10 mg/ml in 25 mM Tris-HCl (pH 8.0).

Crystals of DR1/HA−bispyrrolinone were obtained by hanging drop vapor diffusion. Protein at 10 mg/mL was equilibrated at 18 °C in 10−15% PEG 8000, 100 mM glycine (pH 3.5). Long thin crystals grew within 3 days. Crystals were prepared for liquid nitrogen cooling by adding ethylene glycol gradually to a final concentration 25%.

Data Collection and Processing. X-ray diffraction data from flash cooled crystals of the HLA-DR1/bispyrrolinone complex were collected from a single crystal to 2.5 Å resolution at the BIOCARS station 14-BM-C in Advanced Photon Source using 1 Å wavelength X-rays and Quantum4 CCD detectors. 480 images of 0.5° oscillation were collected. These data were indexed and integrated using DENZO[33] and SCALEPACK.[33] The space group is $C222_1$ with unit cell dimensions $a = 96.51$ Å, $b = 111.75$ Å, $c = 206.06$ Å. Data statistics are in Table 1.

Structure Determination and Refinement. The structure of HLA-DR1/HA−bispyrrolinone complex was determined by molecular replacement using AMoRE.[34] Coordinates of α and β chains of HLA-DR1[5] (PDB accession code 1DHL) were used separately as search models for the molecular replacement. Two sets of α and β chains were found from rotation and translation searches corresponding to two the molecules in the asymmetric unit related by a local 2-fold rotational symmetry axis. After rigid body refinement the correlation coefficient was 0.666, and *R*-factor 0.363 using 10. to 3.5 Å data.

A model of DR1 without bound inhibitor was refined initially and fitted manually into omit maps which were generated by omitting, successively, 10% of the model to avoid bias using the program O.[35] Additionally, density-modified electron density maps were calculated by using iterative solvent flattening, histogram matching, and 2-fold noncrystallographic symmetry (NCS) averaging with DM.[34] After a few cycles of refinement and manual fitting of DR1, a model of the inhibitor could be built into the $2F_o - F_c$ electron density map. The completed model was refined by iterative cycles of manual fitting and positional and group B refinement using CNS (ver. 0.9[36]) with data between 35 and 2.7 Å applying bulk solvent correction and overall anisotropic thermal factor correction in the presence of strong NCS restraints. Restrained individual *B*-factor refinement was included at the final rounds of refinement. The present model has $R_{work} = 0.22$ and $R_{free} = 0.27$.

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