Comparison of Orthorhombic and Monoclinic Crystal Structures of HLA-A2

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The structure of the orthorhombic crystal form of the histocompatibility antigen HLA-A2 has been refined to 2.5 Å resolution. The structure of HLA-A2 had previously been determined to 3.5 Å using data from both orthorhombic and monoclinic crystals (Bjorkman et al. 1987a) and has now been refined to 2.7 Å resolution using the monoclinic data (M.A. Saper et al., in prep.). A comparison of the two structures, which have been independently refined on data from different crystal forms, shows that the structures are very similar. Differences are found only in areas where the electron density is weak (some surface side chains and amino- or carboxy-terminal residues), in those parts of the molecule subject to different packing contacts in the crystals, and in the orientation of a few side chains within the same envelope of electron density. The electron density found in the putative peptide-binding cleft of both HLA-A2 structures is similar in shape and can clearly be seen to reach into several "pockets" around the perimeter of the site.

The determination of the structure of the same molecule twice serves as a control for the level of noise in crystallographic structures, and thus acts as a measure of the significance of structural differences found between different histocompatibility antigens. HLA-Aw68, a second polymorphic class I molecule, crystallizes isomorphously with the orthorhombic form of HLA-A2 (Bjorkman et al. 1985). A comparison of these two alleles permits an assessment of the contribution of amino acid substitutions to the binding specificity of the cleft (T.P.J. Garrett et al., in prep.).

METHODS

Crystallization and data collection on orthorhombic crystals. Crystals formed in hanging drops of 14% polyethylene glycol and 25 mM MES (pH 6.5) as described previously (Bjorkman et al. 1985). They generally measured 150 μm × 150 μm × 60 μm. Data were collected from orthorhombic crystals of HLA-A2 ($P2_12_12_1$, $a = 60.36$ Å, $b = 80.81$ Å, $c = 111.9$ Å) at 4°C, using a Xentronics area detector (Durbin et al. 1986). Data were processed to 2.5 Å using the BUDDHA software package (Blum et al. 1987) together with the CCP4 scaling programs (P. Evans, pers. comm.). The R-factor on intensities was 7.4% to 2.5 Å and 19.1% in the shell from 2.67 Å to 2.5 Å. Data were 93% complete to 2.5 Å and 88% complete (61% > 3σ) in the shell from 2.67 Å to 2.5 Å.

Refinement of a molecular model against orthorhombic data. A molecular model based on the monoclinic data had been partially refined to 2.7 Å using least-squares refinement (M.A. Saper et al., in prep.). A rotation and translation search at 3.5 Å yielded a preliminary transformation from monoclonic to orthorhombic coordinates (Saper et al. 1989). The model was then refined at 2.5 Å using orthorhombic data and a combination of simulated molecular dynamics, least-squares refinement, and manual rebuilding. During an initial round of refinement, data from 5.0 Å to 2.7 Å were used. The program X-PLOR (Brünger 1988) was used to perform energy minimization, overall B-factor refinement, a simulated heating to 1000° K for 0.5 psec, a simulated quenching at 300° K for 0.25 psec, and another round of energy minimization and B-factor refinement. In the second round, the procedure was essentially the same, except that data from 5.5 Å to 2.5 Å were used, B-factors were refined individually, and the simulated heating was modeled at 2500° K. The third round was identical to the second. At this point, simulated heating no longer brought a significant improvement in the R-factor, and subsequent refinement proceeded without it. The final round of refinement therefore involved several stages of manual rebuilding according to difference and $2F_o$-F_c maps, regularization of geometry using FRODO (Jones 1982; Pflugrath et al. 1984), together with X-PLOR energy minimization and individual B-factor refinement to convergence (Brünger 1988). At the end of the process, the resolution range was extended to 6.0 Å to 2.5 Å.

Monoclinic data collection and structure refinement. Data had been collected from monoclinic crystals ($P2_1$, $a = 60.35$ Å, $b = 80.40$ Å, $c = 56.49$ Å, $\beta = 120.42°$) and interpreted at 3.5 Å (Bjorkman et al. 1987a). The model used for comparisons was refined from 6 to 2.7 Å resolution (R-factors = 16.4%) by both geometry- and

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energy-restrained least squares methods (M.A. Saper et al., in prep.).

Comparing the structures. A least-squares fitting algorithm was used to superimpose the α-carbons of the orthorhombic and monoclinic models (Kabsch 1978). The transformation obtained was also used to skew electron density difference maps from the monoclinic to the orthorhombic cell for comparison. A scale factor for comparing the orthorhombic and monoclinic maps was computed by least-squares fitting the electron density values at grid points within 2 Å of atoms in the α_1 and α_2 domains.

In all coordinate comparisons, carboxy-terminal residues 267–270 have been omitted, since there is very little density to support their positions (as is reflected in B-factors above 50 Å^2). Also, during round 3 of refinement, these residues were rebuilt up to 14 Å from their original positions, and these differences dominated the rms statistics.

RESULTS

Orthorhombic Crystal Structure of A2

The structure was refined from an initial R-factor of 37.0% to the current R-factor of 23.0% from 6.0 Å to 2.5 Å. The rms bond length deviation in the current structure is 0.019 Å, and the average deviation of angles is 3.27° (Table 1).

Comparison of the Orthorhombic and Monoclinic A2 Structures

Following a least-squares fit of the α-carbons, the rms difference between all atoms is 0.83 Å. For α-carbons, the rms difference is 0.34 Å. The net C_α rms change during the refinement was similar for domains α_1/α_2 (0.42 Å) and for domains α_3/β_2 (0.37 Å). Also, in the final comparison between the orthorhombic and monoclinic structures, the domains showed a similar overall C_α rms difference of 0.35 Å (α_1/α_2) and 0.31 Å (α_3/β_2).

A list of the 25 α-carbons with differences greater than 1.5*rms includes 14 residues involved in or adjacent to crystal contacts, five of them in the conserved contacts I and II (see below). Four involve amino- or carboxy-terminal residues with B-factors in at least one model of more than 45 Å^2, indicating that they are in areas of the map without good electron density. Six of the seven remaining residues have been affected by different choices in fitting side-chain χ angles to ambiguous density. The final difference, residue 163, appears to be affected by a nearby positive difference electron density peak in the cleft of the orthorhombic structure.

Crystal Contacts

The crystal contacts have been enumerated in Table 2. In the monoclinic crystal, there are four contacts

Table 1. Course of HLA-A2 Orthorhombic Refinement

Round:	start	1	2	3	4
R-factor (6.0–2.5 Å)	0.370	0.277	0.236	0.234	0.230
Geometry (rms deviation from equilibrium):					
bonds (Å)	0.024	0.018	0.019	0.019	0.020
angles (°)	3.0	3.6	3.7	3.3	3.3
peptide bond (°)	1.3	5.8	6.1	5.8	5.7
Average B-factor (Å^2):	16	17	16	24	16
Refinement method[a]:		MD/min	MD/min	build/ MD/min	build/ min
Refinement resolution (Å):		5.0–2.7	5.5–2.5	5.5–2.5	5.5–2.5/ 6.0–2.5
rms change from previous round, following superposition (Å):					
whole molecule (excluding residues 267–270):					
all atoms:		0.73	0.49	0.42	0.21
backbone:		0.40	0.22	0.13	0.072
side chain:		0.92	0.63	0.57	0.29
α_1/α_2:					
all atoms:		0.75	0.46	0.36	0.21
backbone:		0.39	0.22	0.13	0.077
side chain:		0.95	0.58	0.48	0.28
α_3/β_2 m (excluding residues 267–270):					
all atoms:		0.70	0.52	0.48	0.22
backbone:		0.39	0.22	0.14	0.066
side chain:		0.88	0.69	0.65	0.30

[a]Refinement techniques (see text for full details): (MD) simulated molecular dynamics; (min) least-squares energy minimization and B-factor refinement; (build) manual rebuilding or use of the FRODO regularization facility.

Table 2. Crystal Contact Patches, Orthorhombic and Monoclinic

Patch	Form found in	Residues involved	Δ rms backbone (Å)	Δ rms all atoms (Å)	Average B-factor (Å²)[a]
I	ort, mon	17, 18[a], 19, 69, 72, 73[b], 75	0.29	0.83	16
I′	ort, mon	178, 181, 183, 186, 207[a], 209, 238[a], B13, B16, B19, B20	0.31	0.48	13
II	ort, mon	85[a], 121, 122, 135, 136, 137, 138[b]	0.22	0.69	10
II′	ort, mon	219, 222, 226, 249[b], 250[b], 253, 256, 257	0.41	0.73	17
III	mon	84, 142, 149	0.30	0.40	15
III′	mon	105, 106, 108	0.81	1.6	38
IV	mon	176	0.25	1.7	27
IV′	mon	196	0.46	0.91	38
V	ort	127, 131, 154, 155, extra density	0.44	1.5	19
V′	ort	192, 193, 195, 196[c], B74, B96, B98	0.41	0.72	24

Residues preceded by B indicate β_2-microglobulin.
[a]Orthorhombic structure only.
[b]Monoclinic structure only.
[c]Contacts the extra density in HLA-A2 cleft.

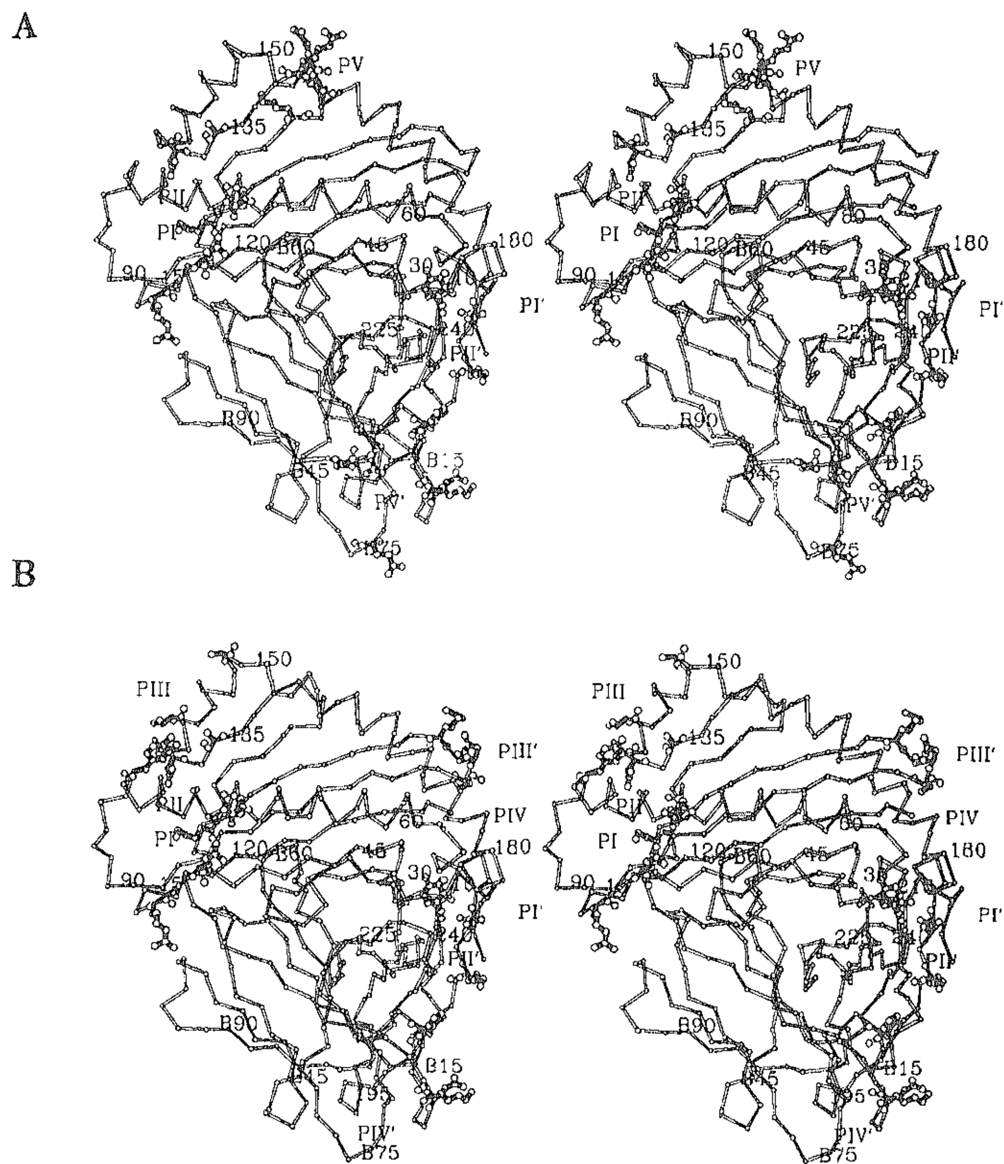
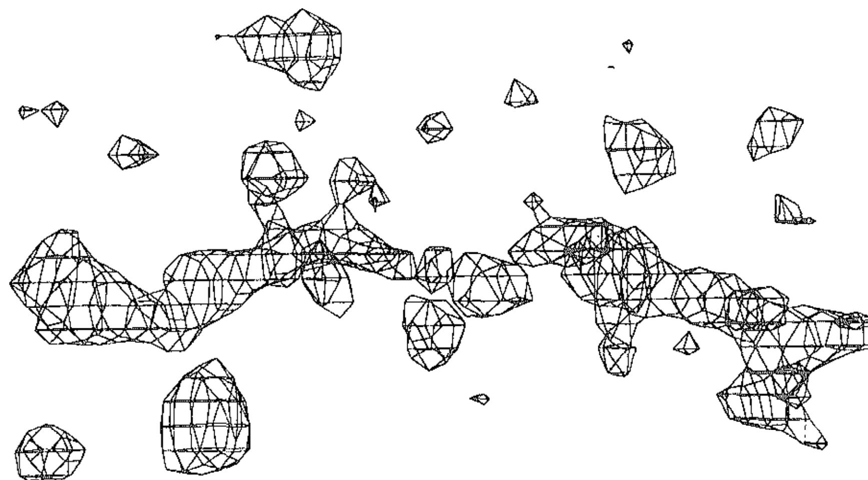


Figure 1. Stereo C_α trace of HLA-A2 with crystal packing contacts for orthorhombic (*A*) and monoclinic (*B*) crystals. Side chains involved in crystal contacts are shown with bold-faced bonds. Residue labels beginning with B indicate β_2-microglobulin. Roman numerals designate contact patches as listed in Table 2.

(eight patches) between symmetry-related molecules within the crystal, which have been labeled so that the two patches forming a contact share the same Roman numeral and are distinguished by being primed or unprimed. There are 39 residues in contacts in the orthorhombic structure and 38 in the monoclinic structure, out of a total of 359 residues in the HLA-A2 heavy chain and β_2-microglobulin. The monoclinic crystal has four contacts (I–IV). The orthorhombic packing shares only contacts I and II with the monoclinic crystal and has one additional contact (V) at the kink in the main α_2 helix.

The relatively conserved contact patch I involves a loop between strands of the $\alpha_1\alpha_2$ β-sheet together with the outside and upper edge of the α_1 helix. Its contact partner, the I′ patch, is a surface spanning the α_3- and β_2-microglobulin domains (Fig. 1). In the two crystal forms, the α-carbon paths of these contact residues are

A



B

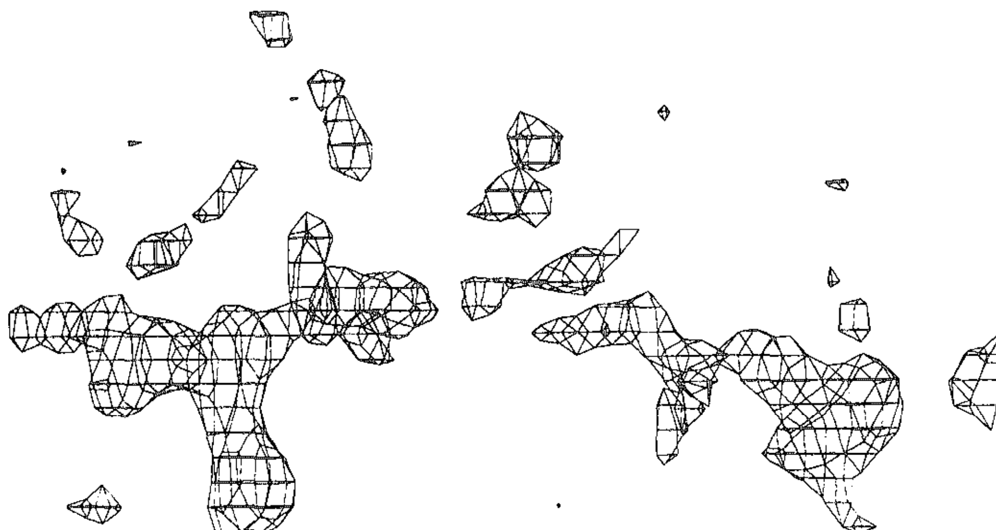


Figure 2. Electron density found in the HLA-A2 cleft. $2F_o$-F_c, calculated phases. (*A*) Orthorhombic data, contoured at 1 σ. (*B*) Monoclinic data, contoured as described in the text.

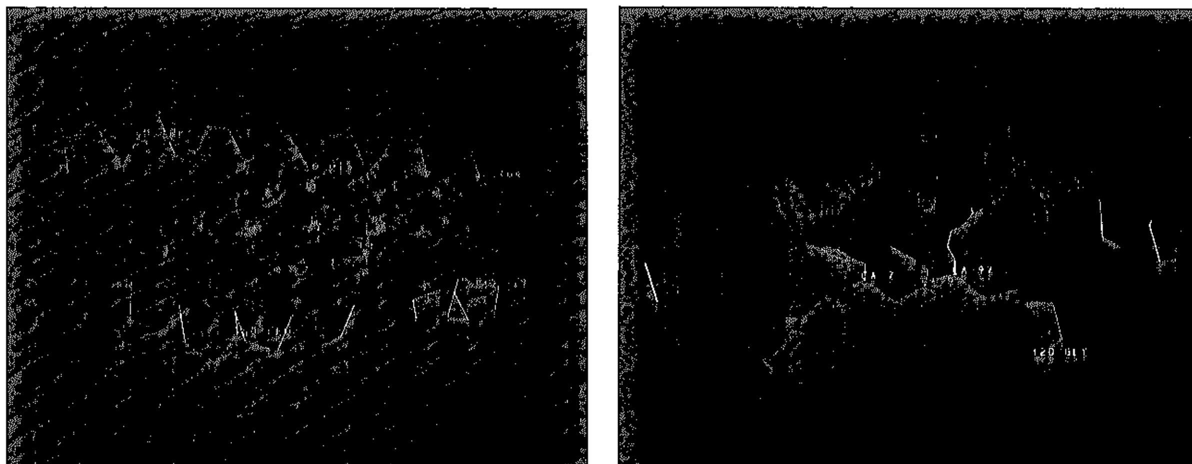


Figure 3. Orthorhombic extra electron density (red) shown in the context of a van der Waals dot surface (blue) of the HLA-A2 site. (*Left*) Top view and (*right*) side view, looking perpendicular to the axes of the α-helices.

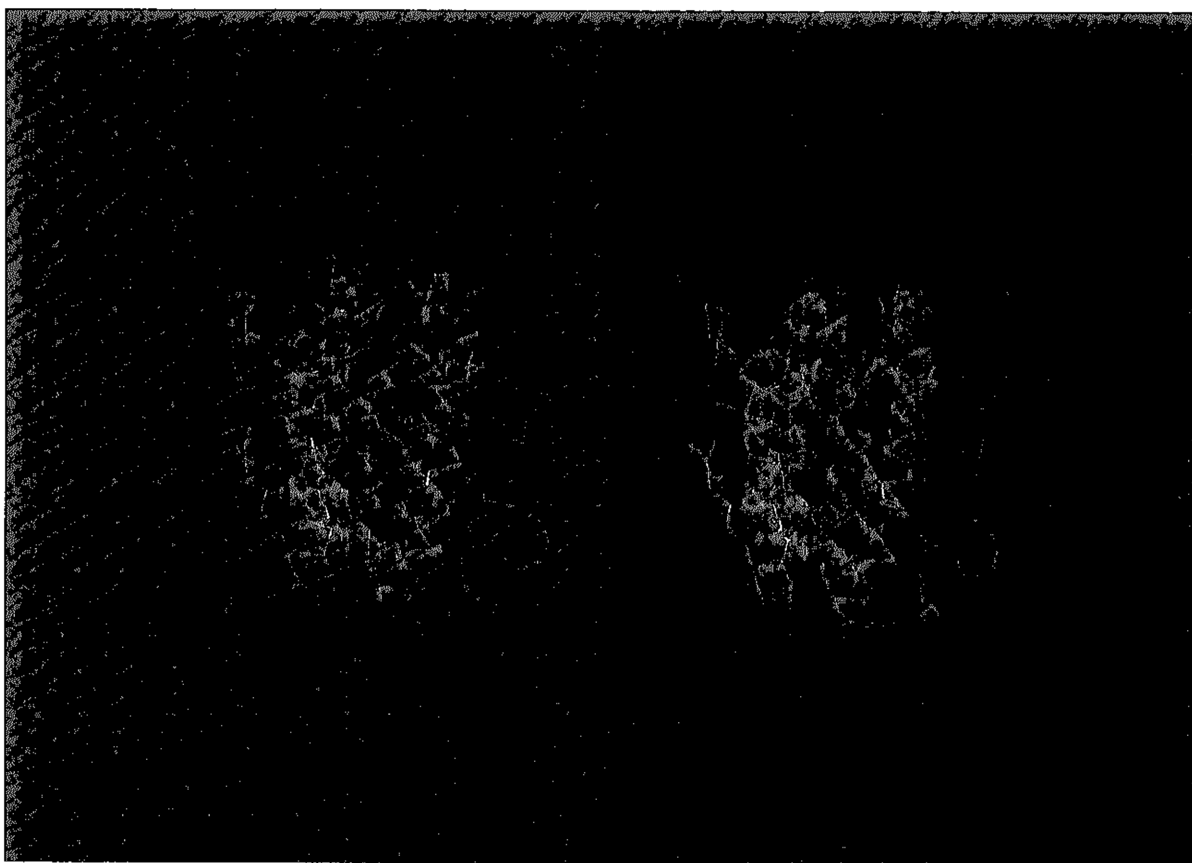


Figure 4. Stereo C_α trace of HLA-A2 α_1 and α_2 domains (blue) with the site residue side chains color-coded by temperature factor. The α_1 helix is to the right, with its amino terminus toward the top of the figure. Blue reflects a low temperature factor (minimum is 2.1 Å^2), and red reflects a high temperature factor (maximum is 30 Å^2).