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Purification, crystallization and preliminary X-ray diffraction analysis of the human major histocompatibility antigen HLA-B*2703 complexed with a viral peptide and with a self-peptide

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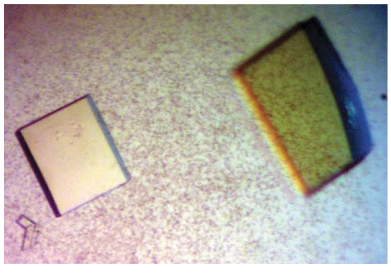


The product of the human leukocyte antigen (HLA) gene *HLA-B*2703* differs from that of the prototypical subtype *HLA-B*2705* by a single amino acid at heavy-chain residue 59 that is involved in anchoring the peptide N-terminus within the A pocket of the molecule. Two B*2703–peptide complexes were crystallized using the hanging-drop vapour-diffusion method using PEG 8000 as a precipitant. The crystals belong to space group $P2_1$ (pVIPR peptide) or $P2_12_12_1$ (pLMP2 peptide). Data sets were collected to 1.55 Å (B*2703–pVIPR) or 2.0 Å (B*2703–pLMP2) resolution using synchrotron radiation. With B*2705–pVIPR as a search model, a clear molecular-replacement solution was found for both B*2703 complexes.

1. Introduction

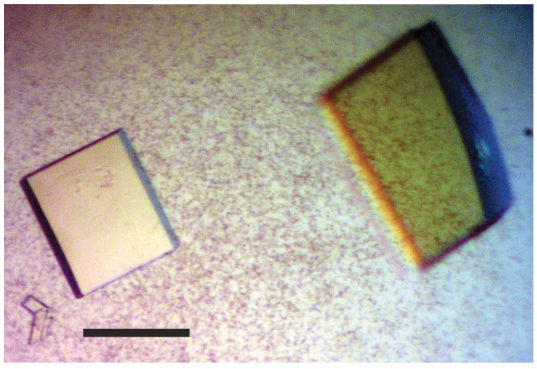
Major histocompatibility complex (MHC; in humans, HLA) class I molecules consist of a highly polymorphic heavy chain (HC) that is non-covalently associated with β_2-microglobulin (β_2m). The HC forms a groove carrying peptides derived from self- or nonself-proteins within the cell. A large number of MHC class I molecules have already been investigated by X-ray crystallography, but the pairwise comparison of very closely related alleles, which additionally may differ in their association with diseases, has only recently been accomplished (Hülsmeyer *et al.*, 2002, 2004, 2005; Macdonald *et al.*, 2003; Webb *et al.*, 2004; Zernich *et al.*, 2004; Fiorillo *et al.*, 2005).

In case of the human MHC class I allele *HLA-B27*, which is very strongly associated with a variety of autoimmune diseases, among them ankylosing spondylitis (AS; Ramos & López de Castro, 2002; Khan & Ball, 2002), peptide presentation has been suspected to play a role in pathogenesis (Benjamin & Parham, 1990; Ramos & López de Castro, 2002). Increased numbers of cytotoxic T lymphocytes (CTL) directed against the self-antigen pVIPR [RRKWRRWHL, derived from vasoactive intestinal peptide type 1 receptor (residues 400–408)] have been found in disease-affected individuals with the *HLA-B*2705* subtype (Fiorillo *et al.*, 2000). About one-sixth of these T cells cross-react with the viral pLMP2 peptide [RRRWRRLTV, derived from latent membrane protein 2 (residues 236–244) of Epstein–Barr virus (EBV); Fiorillo *et al.*, 2000, 2005]. Individuals with another subtype, *HLA-B*2709*, which is not associated with AS and differs from the former only by a single amino acid (His116 instead of Asp116), do not develop CTL responses against the self-peptide pVIPR (Fiorillo *et al.*, 2000), suggesting a *HLA-B27* subtype-dependent connection with AS pathogenesis.

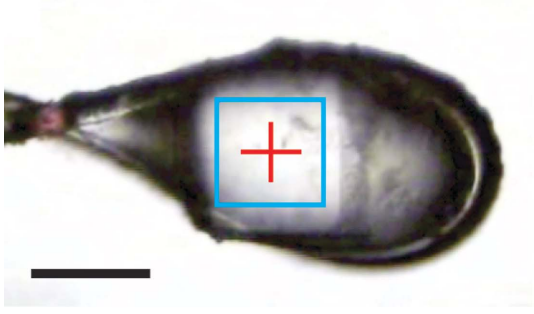
The *HLA-B*2703* subtype is nearly exclusively restricted to black individuals (Rojo *et al.*, 1987; Choo *et al.*, 1988; Gonzalez *et al.*, 2002). Unlike B*2705, it exhibits a questionable association with AS (Ramos & López de Castro, 2002; Khan & Ball, 2002) and its product differs from that of the former subtype by a single amino acid (His59 instead of Tyr59). The consequences of this exchange for peptide binding (Colbert *et al.*, 1994; Boisgérault *et al.*, 1996) or T-cell responses (Villadangos *et al.*, 1994) are entirely distinct from those found for the B*2705/B*2709 pair (Ramos *et al.*, 2002; Fiorillo *et al.*, 2000). HC residue 116 polymorphisms are among the most frequent HLA-B allele exchanges (Reche & Reinherz, 2003) and lead to altered binding of the C-terminal residue of the peptide. In contrast,

of the six HLA class I loci with a current total of 1180 alleles, replacement of Tyr59 occurs only in B*2703 (His59) and B*2717 (Phe59). This exchange is expected to primarily affect the A pocket of the molecule, which binds the peptide N-terminus through hydrogen bonds that are arranged in a highly conserved characteristic pentagonal network (Madden, 1995; Hülsmeyer *et al.*, 2002). The influence of another naturally occurring A-pocket amino-acid exchange on the binding of the peptide N-terminus has already been investigated for B*5101, one of the few subtypes where the common Tyr171 is replaced by His171 (Maenaka *et al.*, 2000). Contrary to residue 59, which is located at the beginning of the α1-helix, residue 171 is part of the end of the α2-helix, opposite residue 59. The His171Tyr exchange results in the complete rearrangement of the above-mentioned pentagonal hydrogen-bonding network within the A pocket (Maenaka *et al.*, 2000).

Determination of the influence of selected polymorphisms on peptide presentation by HLA-B27 subtypes will aid in understanding subtype-dependent differential disease associations (Ramos & López de Castro, 2002; López de Castro *et al.*, 2004). In particular, we addressed the following questions. How are peptides such as pVIPR and pLMP2, whose binding modes have already been determined in the B*2705 and B*2709 subtypes (Hülsmeyer *et al.*, 2004; Fiorillo *et al.*, 2005), bound to B*2703? How does the His59Tyr replacement affect the binding mode of the N-terminal amino acid of the peptide and is its effect comparable to that found for His171 in case of B*5101? Furthermore, can the peptide conformation which characterizes pVIPR and pLMP2 binding in the B*2705 subtype (*i.e.* main-chain φ/ψ torsion angles in α-helical conformation at peptide position p6 instead of the common p4; Hülsmeyer *et al.*, 2004; Fiorillo *et al.*, 2005) also be observed in B*2703? Our study is the first to determine the structural properties of the B*2703 subtype.



(*a*)



(*b*)

Figure 1
Crystals of B*2703–pLMP2. The black bar indicates a length of 80 µm. Crystals of B*2703–pVIPR exhibited the same morphology but were slightly larger (not shown). (*a*) Crystals within the crystallization drop. (*b*) Cooled crystal mounted in a cryoloop at beamline ID14-2 (ESRF) equipped with a mini-diffractometer. The rectangle in cyan represents the size of the X-ray beam.

Table 1
Data-collection statistics of HLA-B*2703–pVIPR and HLA-B*2703–pLMP2.

Values in parentheses refer to the highest resolution shell.

	HLA-B*2703–pVIPR	HLA-B*2703–pLMP2
Space group	$P2_1$	$P2_12_12_1$
Unit-cell parameters		
a (Å)	50.9	50.7
b (Å)	81.6	82.6
c (Å)	65.3	108.3
α (°)	90	90
β (°)	107	90
γ (°)	90	90
Solvent content (%)	58	52
Matthews coefficient† (Å^3 Da^{-1})	2.9	2.6
Resolution (Å)	30.0–1.55 (1.58–1.55)	20.0–2.0 (2.03–2.00)
Unique reflections	70574 (3112)	30890 (1512)
Completeness (%)	95.3 (85.1)	98.3 (97.3)
Redundancy	3.1 (2.7)	4.2 (3.7)
$\langle I/\sigma(I)\rangle$	22.2 (3.4)	16.3 (3.9)
$R_{\rm sym}$‡	0.045 (0.281)	0.074 (0.301)
$R_{\rm merge}$§	0.045 (0.280)	0.070 (0.300)
$R_{\rm r.i.m.}$§	0.054 (0.342)	0.085 (0.346)
$R_{\rm p.i.m.}$§	0.030 (0.193)	0.039 (0.168)

† According to Matthews (1968). ‡ $R_{\rm sym} = \sum_h \sum_i |I_{h,i} - \langle I_h\rangle| / \sum_h \sum_i I_{h,i}$. § According to Weiss (2001).

2. Materials and methods

2.1. Protein preparation

The peptides pVIPR (RRKWRRWHL) and pLMP2 (RRRWRRLTV) were synthesized by the solid-phase method and purified by Alta Bioscience (Birmingham, England). The extracellular region of the B*2703 heavy chain (the clone was generated by *in vitro* mutagenesis from a B*2705 clone) and β_2m were expressed separately as inclusion bodies in *Escherichia coli*, dissolved in 50%(*w*/*v*) urea and the HLA-B27–peptide complexes were reconstituted for 14 d at 277 K as described previously (Garboczi *et al.*, 1992) with slight modifications. Briefly, unfolded HC (12 mg), β_2m (10 mg) and 4 mg of either pVIPR or pLMP2 were rapidly injected into 400 ml of refolding buffer (400 m*M* arginine–HCl, 2 m*M* EDTA, 5 m*M* reduced glutathione, 0.5 m*M* oxidized glutathione and 100 m*M* Tris–HCl pH 7.5). The mixture was concentrated using Amicon Ultra-15 devices and the complexes were isolated by size-exclusion chromatography and used for crystallization at concentrations of 13–15 mg ml^{-1} in 20 m*M* Tris–HCl, 150 m*M* NaCl, 0.01% sodium azide pH 7.5.

2.2. Crystallization and data collection

All crystallization trials were performed in a hanging-drop vapour-diffusion setup at 291 K (1.5 µl protein solution and 1.5 µl precipitant solution), employing the previously described conditions (Hülsmeyer *et al.*, 2002, 2005). Crystal formation for both complexes was optimized by varying the PEG concentration in the precipitant solution [18–28%(*w*/*v*) PEG 8000, 100 m*M* Tris–HCl pH 7.0]. To increase the crystal size, streak-seeding was applied by passing a cat whisker through each crystallization drop in the screens. After 4 d, crystals of B*2703–pVIPR grew as plates and maximum dimensions of 200 × 100 × 10 µm were obtained at 18%(*w*/*v*) PEG 8000. The crystals of B*2703–pLMP2 had identical morphology, but were always smaller than those from B*2703–pVIPR, with approximate dimensions of 80 × 80 × 5 µm obtained at a PEG 8000 concentration of 22%(*w*/*v*) (Fig. 1).

Prior to data collection, the crystals in the crystallization drops were cryoprotected by stepwise increase of glycerol and PEG 8000 to final concentrations of 10 and 21%, respectively. An initial data set for B*2703–pVIPR was collected at the Protein Structure Factory

beamline BL14.2 of the Free University Berlin at Berliner Elektronenspeicherring–Gesellschaft für Synchrotronstrahlung mbH (BESSY, Berlin, Germany). Data sets with the highest diffraction limit were collected at the European Synchrotron Radiation Facility (ESRF, Grenoble, France), beamline ID 14-2, at a wavelength of 0.933 Å at 100 K. This beamline is equipped with a novel MD2M mini-diffractometer and an ADSC-Q4 (Area Detector Systems Corperation) CCD detector. The mini-diffractometer simplified the precise centring of the small crystals of B*2703–pLMP2. Visual inspection of the diffraction pattern from crystals of B*2703–pLMP2 clearly showed elongated spots. Flash annealing extended the diffraction limit from the initial 2.4 to 2.0 Å and improved the spot shape. For annealing, the cryostream was blocked with a canteen card for approximately 5 s until melting became visible; the card was then quickly removed to allow re-cooling.

Data were processed with *DENZO* and scaled with *SCALEPACK* (Otwinowski & Minor, 1997). The results are summarized in Table 1. The B*2703–pVIPR crystals belong to the monoclinic space group $P2_1$, whereas the crystals of B*2703–pLMP2 belong to an orthorhombic space group. Molecular replacement was performed using coordinates of the high-resolution crystal structure of B*2705–pVIPR (Hülsmeyer *et al.*, 2004; PDB code 1ogt; water molecules and peptide were omitted) as a search model. Unambiguous solutions of both structures were found using the program *PHASER* (Storoni *et al.*, 2004) with diffraction data in the resolution range 20–3 Å. Initial $F_o - F_c$ difference maps revealed the undoubted presence of the peptides when inspected with the program *O* (Jones *et al.*, 1991).

3. Results

The B*2703–pVIPR and B*2703–pLMP2 complexes were crystallized and crystal formation was optimized using streak-seeding techniques (Fig. 1). X-ray diffraction analysis revealed that the crystals of the two complexes were not isomorphous (Table 1), contrary to our expectation from the experiments with the B*2705 and B*2709 subtypes, which crystallized in the $P2_1$ space group when complexed with pVIPR and pLMP2 (Hülsmeyer *et al.*, 2004; Fiorillo *et al.*, 2005). B*2703–pLMP2 crystallized in space group $P2_12_12_1$, which has previously been observed in crystals of B*2705 and B*2709 [each in complex with two peptides, m9 (Hülsmeyer *et al.*, 2002) or TIS (Hülsmeyer *et al.*, 2005)]. This finding indicates that B*2703–pLMP2 as well as B*2705 and B*2709 complexed with m9 or TIS adopt similar conformations. The crystals diffracted to 1.55 Å (B*2703–pVIPR) and 2.0 Å (B*2703–pLMP2). Therefore, a detailed comparison will be possible not only between the two B*2703–peptide complexes, but also between them and peptides complexed with B*2705, B*2709 and B*5101. Further refinement of both B*2703–peptide complexes is in progress.

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References

Benjamin, R. & Parham, P. (1990). *Immunol. Today*, **11**, 137–142.
Boisgérault, F., Tieng, V., Stolzenberg, M. C., Dulphy, N., Khalil, I., Tamouza, R., Charron, D. & Toubert, A. (1996). *J. Clin. Invest.* **98**, 2764–2770.
Choo, S. Y., St John, T., Orr, H. T. & Hansen, J. A. (1988). *Hum. Immunol.* **21**, 209–219.
Colbert, R. A., Rowland-Jones, S. L., McMichael, A. J. & Frelinger, J. A. (1994). *Immunity*, **1**, 121–130.
Fiorillo, M. T., Maragno, M., Butler, R., Dupuis, M. L. & Sorrentino, R. (2000). *J. Clin. Invest.* **106**, 47–53.
Fiorillo, M. T., Rückert, C., Hülsmeyer, M., Sorrentino, R., Saenger, W., Ziegler, A. & Uchanska-Ziegler, B. (2005). *J. Biol. Chem.* **280**, 2962–2971.
Garboczi, D. N., Hung, D. T. & Wiley, D. C. (1992). *Proc. Natl Acad. Sci. USA*, **89**, 3429–3433.
Gonzalez, S., Garcia-Fernandez, S., Martinez-Borra, J., Blanco-Gelaz, M. A., Rodrigo, L., Sanchez del Rio, J., López-Vazquez, A., Torre-Alonso, J. C. & López-Larrea, C. (2002). *Hum. Immunol.* **63**, 673–676.
Hülsmeyer, M., Fiorillo, M. T., Bettosini, F., Sorrentino, R., Saenger, W., Ziegler, A. & Uchanska-Ziegler, B. (2004). *J. Exp. Med.* **199**, 271–281.
Hülsmeyer, M., Hillig, R. C., Volz, A., Rühl, M., Schröder, W., Saenger, W., Ziegler, A. & Uchanska-Ziegler, B. (2002). *J. Biol. Chem.* **277**, 47844–47853.
Hülsmeyer, M., Welfle, K., Pöhlmann, T., Misselwitz, R., Alexiev, U., Welfle, H., Saenger, W., Uchanska-Ziegler, B. & Ziegler, A. (2005). *J. Mol. Biol.* **346**, 1367–1379.
Jones, T. A., Zou, J. Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.
Khan, M. A. & Ball, E. J. (2002). *Best Pract. Res. Clin. Rheumatol.* **16**, 675–690.
López de Castro, J. A., Alvarez, I., Marcilla, M., Paradela, A., Ramos, M., Sesma, L. & Vazquez, M. (2004). *Tissue Antigens*, **63**, 424–445.
Macdonald, W. A., Purcell, A. W., Mifsud, N. A., Ely, L. K., Williams, D. S., Chang, L., Gorman, J. J., Clements, C. S., Kjer-Nielsen, L., Koelle, D. M., Burrows, S. R., Tait, B. D., Holdsworth, R., Brooks, A. G., Lovrecz, G. O., Lu, L., Rossjohn, J. & McCluskey, J. (2003). *J. Exp. Med.* **198**, 679–691.
Madden, D. R. (1995). *Annu. Rev. Immunol.* **13**, 587–622.
Maenaka, K., Maenaka, T., Tomiyama, H., Takiguchi, M., Stuart, D. I. & Jones, E. Y. (2000). *J. Immunol.* **165**, 3260–3267.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Ramos, M. & López de Castro, J. A. (2002). *Tissue Antigens*, **60**, 191–205.
Ramos, M., Paradela, A., Vazquez, M., Marina, A., Vazquez, J. & López de Castro, J. A. (2002). *J. Biol. Chem.* **277**, 28749–28756.
Reche, P. A. & Reinherz, E. L. (2003). *J. Mol. Biol.* **331**, 623–641.
Rojo, S., Aparicio, P., Hansen, J. A., Choo, S. Y. & López de Castro, J. A. (1987). *J. Immunol.* **139**, 3396–3401.
Storoni, L. C., McCoy, A. J. & Read, R. J. (2004). *Acta Cryst.* D**60**, 432–438.
Villadangos, J. A., Galocha, B., Garcia-Hoyo, R., Lopez, D., Garcia, F. & López de Castro, J. A. (1994). *Eur. J. Immunol.* **24**, 2548–2555.
Webb, A. I., Borg, N. A., Dunstone, M. A., Kjer-Nielsen, L., Beddoe, T., McCluskey, J., Carbone, F. R., Bottomley, S. P., Aguilar, M. I., Purcell, A. W. & Rossjohn, J. (2004). *J. Immunol.* **173**, 402–409.
Weiss, M. (2001). *J. Appl. Cryst.* **34**, 130–135.
Zernich, D., Purcell, A. W., Macdonald, W. A., Kjer-Nielsen, L., Ely, L.K., Laham, N., Crockford, T., Mifsud, N. A., Bharadwaj, M., Chang, L., Tait, B. D., Holdsworth, R., Brooks, A. G., Bottomley, S. P., Beddoe, T., Peh, C. A., Rossjohn, J. & McCluskey, J. (2004). *J. Exp. Med.* **200**, 13–24.