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Crystallization and preliminary X-ray crystallographic analysis of the rhesus macaque MHC class I molecule Mamu-B*17 complexed with an immunodominant SIVmac239 Env epitope

Long-term nonprogression during simian immunodeficiency virus (SIV) infection has been strongly associated with the major histocompatibility complex (MHC) class I allele Mamu-B*17. Here, a complex of rhesus macaque Mamu-B*17 with rhesus macaque β_2 -microglobulin (β_2 m) and an immunodominant peptide (SIVmac239 Env241–251; LRCNDTNYSGF; Env LF11) derived from the SIV Env protein was crystallized by the hanging-drop method using PEG 3350 as a precipitating agent. The crystals belonged to the primitive monoclinic space group *P*2₁, with unit-cell parameters $a = 68.3$, $b = 45.0$, $c = 81.5$ Å, $\beta = 96.5^\circ$. Assuming the presence of one molecule in the asymmetric unit, the Matthews coefficient and solvent content were calculated to be 2.96 Å³ Da^{−1} and 58.5%, respectively.

1. Introduction

It is important to find a well defined animal model for the study of human immunodeficiency virus (HIV) infection of humans. At present, SIV infection of the rhesus macaque represents perhaps the best animal model for HIV infection of humans (Johnson, 1996; Stott & Almond, 1995). At the molecular level, it has been reported that the binding motif of the peptide to major histocompatibility complex (MHC) class I molecules is generally conserved between humans and rhesus macaques (Dzuris *et al.*, 2000). The immune systems of rhesus macaques and humans are also similar. Many macaque genes that encode proteins that are important in the function of the immune system are remarkably similar to their human homologues. Specifically, homologues of the human MHC class I (Boyson *et al.*, 1996; Watkins, 1995; Stott & Almond, 1995), class II (Slienderregt *et al.*, 1992) and TCR genes (Levinson *et al.*, 1992; Bontrop *et al.*, 1995) are all found in macaques. Notably, the infection of macaques with SIV results in an AIDS-like illness similar to that of humans infected with HIV (Allen *et al.*, 1998; Gotch *et al.*, 1993). The use of the Indian rhesus macaque as a model for HIV infection confirms the important role of MHC class I molecules in mediating antiviral immune responses and has led to the development of molecular class I typing methods and the application of synthetic peptide-binding assays for macaque MHC molecules.

HIV infection leads to acute high-level viraemia, which is subsequently reduced to a set-point viral load. Without therapy, most patients experience a subsequent increase in viral load and ultimately the development of AIDS. However, some HIV-infected individuals remain clinically healthy for years and are called long-term non-progressors (LTNPs). Elite controllers (ECs) are a subset of LTNPs that control the HIV replication level in the plasma below a detectable threshold. Some MHC class I alleles have been associated with LTNPs or ECs (Kaslow *et al.*, 1996; Migueles *et al.*, 2000; Hendel *et al.*, 1999; Magierowska *et al.*, 1999). HLA-B*57 and HLA-B*27 are two typical EC alleles in humans. A similar phenomenon has been observed in rhesus macaques (Loffredo *et al.*, 2007; Yant *et al.*, 2006). Mamu-B*17 is one of the protective alleles in macaques, and this allele is present in over 12% of the captive-bred Indian rhesus macaque population, making this class I molecule a good candidate for extensive characterization (Yant *et al.*, 2006). The molecular features of the peptide ligands bound by Mamu-B*17 have been characterized *in vitro* (Mothé *et al.*, 2002). The epitope (LF11) that we

have chosen in this study is derived from the SIVmac239 Env protein, which plays an important role in the initial steps of infection and also in viral assembly at a later step of infection. Effector CD8⁺ T cells (CTLs) are implicated in viral control because the T-cell antigen receptors (TCRs) on CD8⁺ T cells recognize the complexes of viral peptides and class I HLA molecules presented on the surface of infected cells. ELISPOT (enzyme-linked immunosorbent spot) and ICS (intracellular cytokine staining) assays with PBMCs (peripheral blood mononuclear cells) from SIV-infected macaques have revealed that the Env LF11 peptide has the capacity to elicit a CTL response (Mothé *et al.*, 2002).

2. Materials and methods

2.1. Expression of Mamu-B*17 and β_2m proteins as inclusion bodies

The Mamu-B*17 (residues 1–276) and rhesus macaque β_2m -microglobulin (β_2m ; residues 1–99) coding sequences were inserted into pET-21a vectors and the plasmids were transformed into 50 μ l of competent *Escherichia coli* BL21 cells. A positive isolate was used to inoculate 1 l warm BL medium (10 g l⁻¹ tryptone, 10 g l⁻¹ NaCl, 5 g l⁻¹ yeast extract) containing 50 mg l⁻¹ ampicillin. The transformed cells were induced using 1 mM isopropyl β -D-1-thiogalactopyranoside when the OD₆₀₀ reached 0.6, cultured at 310 K for 16 h and harvested by centrifugation (8000g, 10 min, 277 K). The cell pellet was resuspended in 20 ml phosphate-buffered saline buffer. The cells were broken by sonication; after centrifugation (8000g, 10 min, 277 K) the pellet was treated with Triton wash buffer (0.5% Triton X-100, 50 mM Tris pH 8, 300 mM NaCl, 10 mM EDTA, 10 mM DTT). The protein in the form of inclusion bodies was washed three times and dissolved to 30 mg ml⁻¹ in guanidine buffer (6 M guanidine, 10% glycerol, 50 mM Tris pH 8, 10 mM EDTA, 100 mM NaCl, 10 mM DTT).

2.2. Refolding and purification of the Mamu-B*17–Env LF11 complex

The synthetically prepared peptide (SIVmac239 Env241–251; Env LF11) was dissolved in DMSO. 90 mg Mamu-B*17 heavy chain, 30 mg β_2m and 5 mg peptide were subsequently injected into 1 l

refolding buffer (100 mM Tris pH 8, 400 mM L-arginine-HCl, 2 mM EDTA, 5 mM reduced glutathione, 0.5 mM oxidized glutathione). After stirring at 277 K for 24–48 h, the soluble refolded portion was concentrated using 10 kDa cutoff centrifugal filter units and was then purified by chromatography on a Superdex 75 size-exclusion column (Amersham Pharmacia Biotech) eluted with 20 mM Tris pH 8, 50 mM NaCl. The chromatographic profile showed only one peak, which corresponded to the refolded complex (42 kDa; Fig. 1).

2.3. Crystallization and data collection

The Mamu-B*17–Env LF11 complex was concentrated to 10 mg ml⁻¹ in 20 mM Tris pH 8, 50 mM NaCl buffer using 10 kDa cutoff centrifugal filter units. Crystallization screening was initiated

Figure 1
Purification of the refolded complex of the Mamu-B*17 heavy chain with rhesus macaque β_2m and the Env LF11 peptide by FPLC Superdex 75 size-exclusion chromatography. The gel-filtration profile of the refolded product and reduced SDS-PAGE gel (15%) of the purified protein (inset) are shown.

(a)

(b)

Figure 2
(a) Typical crystal of Mamu-B*17 complexed with Env LF11. The length of the scale bar is 0.3 mm. (b) X-ray diffraction image from the crystal of Mamu-B*17 complexed with Env LF11. The black ring represents 2.1 Å resolution.

Table 1

Crystal data and intensity statistics.

Values in parentheses are for the outermost resolution shell.

Space group	<i>P2</i>
Unit-cell parameters (Å, °)	<i>a</i> = 68.3, <i>b</i> = 45.0, <i>c</i> = 81.5, β = 96.5
Resolution range (Å)	30.00–2.55 (2.64–2.55)
No. of observed reflections	112011
No. of unique reflections	16196
Completeness (%)	98.3 (97.0)
<i>R</i> _{merge} † (%)	10.4 (46.1)
Mean <i>I</i> /σ(<i>I</i>)	22.0 (4.7)
Multiplicity	6.9 (6.9)

† $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th observation of unique reflection *hkl* and $\langle I(hkl) \rangle$ is the average over symmetry-related observations of unique reflection *hkl*.

with the Index screen (Hampton Research) using drops consisting of 1 µl protein solution and 1 µl crystallization buffer and the hanging-drop method. Plates were incubated at 291 K and analyzed after 24 h, 48 h and one week. Crystals were observed in Index condition Nos. 71 and 72 after 48 h. The crystals were flash-cooled in liquid nitrogen in CryoLoops and a working data set was collected to 2.55 Å resolution from crystals grown in Index condition No. 72 [0.2 M sodium chloride, 0.1 M HEPES pH 7.5, 25%(w/v) polyethylene glycol 3350]. Data collection was performed in-house on a Rigaku R-AXIS IV⁺⁺ image plate using a Rigaku MicroMax-007 rotating-anode X-ray generator operated at 40 kV and 20 mA (Cu Kα; λ = 1.5418 Å). Data were processed and scaled using *HKL-2000* (Otwinowski & Minor, 1997).

3. Results and discussion

Mamu-B*17 heavy chain and rhesus macaque β₂m were expressed as inclusion bodies. After refolding and purification, pure Mamu-B*17–Env LF11 complex was obtained, as verified by SDS–PAGE (Fig. 1). The final yield of the pure complex protein was 10 mg starting with a 1 l cell culture expressing Mamu-B*17 heavy chain and a 1 l cell culture expressing β₂m. The complex was concentrated to 10 mg ml^{−1} in 20 mM Tris pH 8, 50 mM NaCl buffer for crystallization. Crystals of the Mamu-B*17–Env LF11 complex were grown using the hanging-drop vapour-diffusion method (Fig. 2*a*). We believe the complex includes the Env LF11 peptide because to our knowledge it is not possible to produce a stable refolded MHC molecule without the addition of peptide. Data were collected to 2.55 Å resolution using a Rigaku MicroMax-007 generator (Fig. 2*b*). Selected data statistics are shown in Table 1. The space group was *P2*. The choice between *P2*

and *P2*₁ was made by analysis of the intensity-to-noise ratio of the (0, 2*n*, 0) and (0, 2*n* + 1, 0) reflections. The gel-filtration experiment showed that the rhesus macaque MHC class I molecule Mamu-B*17 complexed with an SIVmac239 Env peptide existed as a monomer in solution. Assuming one monomer per asymmetric unit the Matthews coefficient (*V*_M) is 2.96 Å³ Da^{−1}, corresponding to a solvent content of 58.5%.

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