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X-ray crystallographic characterization of rhesus macaque MHC Mamu-A*02 complexed with an immunodominant SIV-Gag nonapeptide

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Simian immunodeficiency virus (SIV) in the rhesus macaque is regarded as a classic animal model, playing a crucial role in HIV vaccine strategies and therapeutics by characterizing various cytotoxic T-lymphocyte (CTL) responses in macaque monkeys. However, the availability of well documented structural reports focusing on rhesus macaque major histocompatibility complex class I (MHC I) molecules remains extremely limited. Here, a complex of the rhesus macaque MHC I molecule (Mamu-A*02) with human β_2m and an immunodominant SIV-Gag nonapeptide, GESNLKSLY (GY9), has been crystallized. The crystal diffracts X-rays to 2.7 Å resolution and belongs to space group $C2$, with unit-cell parameters a = 124.11, b = 110.45, c = 100.06 Å, and contains two molecules in the asymmetric unit. The availability of the structure, which is being solved by molecular replacement, will provide new insights into rhesus macaque MHC I (Mamu-A*02) presenting pathogenic SIV peptides.

1. Introduction

Human immunodeficiency virus (HIV) has been found to be causative of human acquired immunodeficiency syndrome (AIDS) and has caused the loss of hundreds of thousands of human lives worldwide over the last 25 y. It has been widely accepted that AIDS remains one of the most disastrous threats to human health in the world and that there is an urgent and great need for prevention strategies and therapeutics (Watanabe *et al.*, 1994; Harper, 1999). Based on evidence concerning their genetic, antigenic and biological properties, simian immunodeficiency viruses (SIV) are regarded as the closest known relatives of HIV; various infection experiments on rhesus macaques have also resulted in an AIDS-like disease, which in turn further confirms the relationship between SIV and HIV (McClure *et al.*, 1990). The infection of rhesus macaques with SIV has been developed as one of the best animal models for investigating the pathogenesis of HIV-like viruses and newly developed antiretroviral drugs or vaccines have been evaluated in macaque monkeys (McClure *et al.*, 1990). It has been found that SIV infection shares similar phenomena to HIV infection, which is presented by T-cell immunity, especially cytotoxic T-lymphocyte (CTL) responses (Desrosiers *et al.*, 1989; Gotch *et al.*, 1993; Allen *et al.*, 1998). Moreover, macaque and human major histocompatibility complex class I (MHC I) molecules have been revealed to bind to peptides derived from similar fragments of the Gag and Env proteins of HIV and AIV (Gotch *et al.*, 1993). Undoubtedly, knowledge obtained from the rhesus macaque immunological response to SIV will definitely facilitate to demystify the enigma underlying the pathways of HIV infection. However, knowledge of the structural basis of the binding of rhesus macaque MHC I molecules to their specific peptides remains relatively limited. Here, we report a complex crystal of the rhesus macaque MHC I molecule (Mamu-A*02) with human β_2m and an immunodominant SIV-Gag nonapeptide, GESNLKSLY (GY9), which diffracts X-rays to 2.7 Å. The structure, which is being solved by molecular replacement, will provide new insights into Mamu-A*02 presenting pathogenic SIV peptides.

2. Materials and methods

2.1. Development of the recombinant prokaryotic expression plasmids

A 276-amino-acid portion of the extra-domain of the Mamu-A*02 heavy chain, with its signal peptide deleted and the transmembrane region truncated, was amplified from the eukaryotic expression plasmid, a gift from Dr David Watkins, using primers Mamu-A*02-F1, 5′-CCAA*CATATG*GGCTCTCACTCCATGAGGTAT-3′ (*Nde*I site in italics) and Mamu-A*02-R, 5′-CCG*CTCGAG*TTACGGC-TCCCATCTCAGGGT-3′ (*Xho*I site in italics). The polymerase chain reaction (PCR) mixture (25 µl) consisted of 0.2 p*M* forward and reverse primers, 25 µ*M* dNTP, 2 ng plasmid template and 1 U of *Pfu* DNA polymerase in the corresponding buffer. The PCR amplification was performed in a thermocycler (Bio-Rad) and consisted of 3 min of pre-incubation at 368 K followed by 30 cycles of 0.5 min at 368 K, 1.0 min at 331 K and 3.0 min at 345 K. As a final extension it was kept for 8 min at 345 K. The purified PCR product was digested and then inserted directionally into the prokaryotic expression vector pET21a(+) (Novagen) *via* the *Nde*I and *Xho*I restriction sites (introduced by the PCR primers). Finally, the acquired recombinant plasmid designated for pET21::Mamu-A*02 was verified by direct DNA sequencing and used for expression of Mamu-A*02 heavy chain in *Escherichia coli* strain BL21 (DE3). The prokaryotic expression plasmid named pGMT7::β_2m, for production of the human β_2m protein, was constructed previously (Willcox *et al.*, 1999).

2.2. Production of Mamu-A*02 and β_2m proteins as inclusion bodies

Both recombinant proteins, Mamu-A*02 and β_2m, were expressed as inclusion bodies in *E. coli*, lysed employing sonication and then collected by centrifugation at 12 600 rev min^{-1}. The pellet was then washed three times with a solution of 20 m*M* Tris–HCl, 100 m*M* NaCl, 1 m*M* EDTA, 1 m*M* DTT and 0.5% Triton-100 (Zhou *et al.*, 2004; Chu, Luo, Gao *et al.*, 2005).

2.3. Preparation of the Mamu-A*02–β_2m–GY9 complex

The preparation of the Mamu-A*02–β_2m–GY9 complex was conducted using the protocol previously described by Garboczi *et al.* (1992) with some modifications introduced in our laboratory (Zhou *et al.*, 2004; Chu, Lou, Gao *et al.*, 2005). Briefly, the Mamu-A*02 heavy chain and β_2m inclusion bodies were separately dissolved in a solution of 10 m*M* Tris–HCl pH 8.0 and 8.0 *M* urea. The synthetically prepared GY9 peptide (GSENLKSLY; SIV Gag amino acids 71–79; Vogel *et al.*, 2002) was dissolved in dimethyl sulfoxide (DMSO). Mamu-A*02 heavy chain, β_2m light chain and the nine-peptide GY9 were combined in a molar ratio of 1:1:3 and refolded by the gradual dilution method. After about 24 h of incubation at 277 K, the remaining soluble portion of the complex was concentrated and subsequently purified by size-exclusion chromatography on a Superdex 200 (Amersham Biosciences) column.

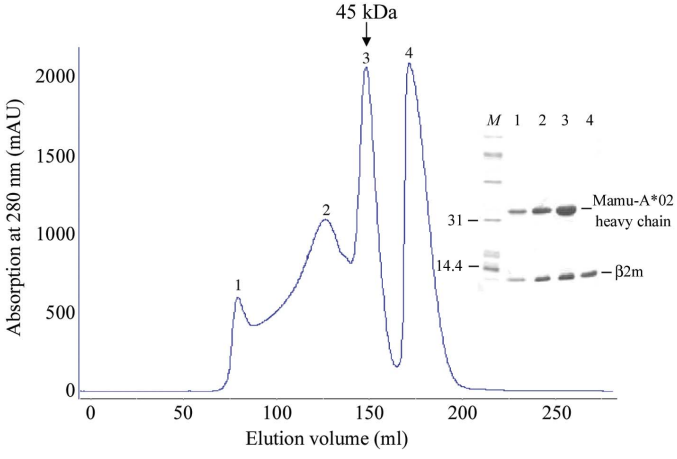

Figure 1
Purification of the refolded Mamu-A*02–β_2m–GY9 complex by FPLC Superdex G75 gel filtration. Lane *M*, protein molecular-weight standards (kDa); lanes 1, 2, 3 and 4, proteins collected from peaks 1, 2, 3 and 4, respectively.

Table 1
X-ray diffraction data and processing statistics.

Unit-cell parameters (Å, °)	a = 124.11, b = 110.45, c = 100.06, α = 90.00, β = 114.41, γ = 90.00
Space group	*C*2
Resolution (Å)	50–2.7 (2.8–2.7)
No. of reflections	248133
Unique reflections	33701
Completeness (%)	99.7 (100)
Average $I/\sigma(I)$	8.2 (4.5)
R_{merge}	0.176 (0.402)
Redundancy	7.36 (7.31)

2.4. Crystallization of the Mamu-A*02–β_2m–GY9 complex

The purified protein complex (∼45 kDa) was dialyzed against crystallization buffer (20 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl) and concentrated to 16 mg ml^{-1}. Crystallization trials were carried out with Crystal Screens I and II (Hampton Research) at 291 K using the hanging-drop method. A drop containing equal volumes (1 µl each) of protein solution (16 mg ml^{-1}) and reservoir solution was placed over a well containing 200 ml reservoir solution using a VDX plate (HR3-142). Crystals were obtained in 1–2 months with solution No. 13 of Crystal Screen II (0.1 *M* sodium acetate pH 4.6, 0.2 *M* ammonium sulfate and 30% polyethene glycol monomethyl ether 20 000) and solution No. 22 of Crystal Screen II [0.1 *M* MES pH 6.5, 12%(*w*/*v*) polyethylene glycol]. The conditions yielding crystals were further optimized by variation of the precipitant gradient, the protein concentration and the pH of the buffer. Finally, crystals suitable for data collection were harvested within 6–8 weeks from the following two improved conditions: (i) 0.1 *M* Tris–HCl pH 5.5, 0.12 *M* ammonium sulfate and 38% polyethene glycol monomethyl ether 20 000 and (ii) 0.1 *M* MES pH 6.5, 0.32 *M* ammonium sulfate and 38% polyethene glycol monomethyl ether 20 000.

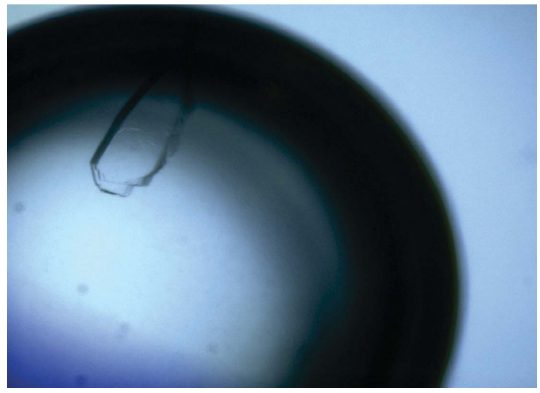

Figure 2
Typical crystal of the Mamu-A*02–β_2m–GY9 complex.

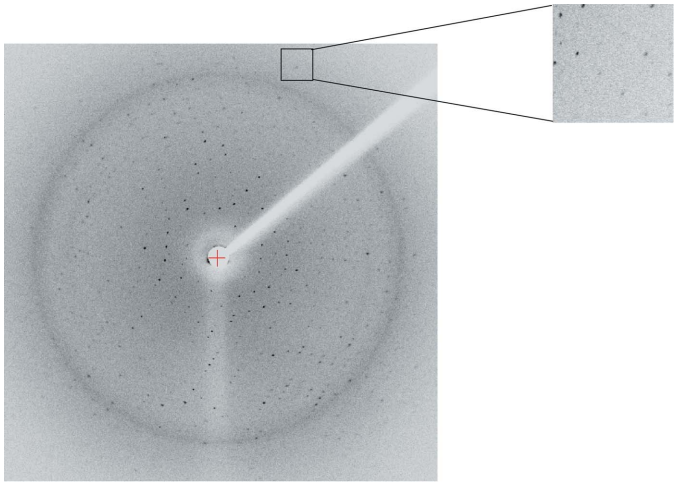

Figure 3
Diffraction pattern of the Mamu-A*02–β_2m–GY9 complex at 2.7 Å.

2.5. Collection and processing of the crystal diffraction data

Data collection from the Mamu-A*02 complex was carried out in-house on a Rigaku MicroMax007 rotating-anode X-ray generator operated at 40 kV and 20 mA (Cu $K\alpha$; λ = 1.5418 Å) which was equipped with Osmic mirrors and an R-AXIS VII** image-plate detector. The crystals were mounted in nylon loops and flash-cooled in a cold nitrogen-gas stream at 100 K using an Oxford Cryosystem with reservoir solution as the cryoprotectant. A complete data set was collected to 2.7 Å. Data were indexed, integrated and scaled using *DENZO* and *SCALEPACK* as implemented in *HKL*2000 (Otwinowski & Minor, 1997).

3. Results and discussion

The complex refolding resulted in yields of approximately 15% of the correctly folded complex (~45 kDa), which could be purified to homogeneity by Superdex G-75 size-exclusion chromatography (Fig. 1). The chromatographic profile showed four peaks corresponding to the aggregate products (peak 1), the putative dimer (peak 2), the target refolded protein complex (~45 kDa; peak 3) and surplus β_2m (peak 4). The refolded complex was further confirmed by SDS–PAGE (seen in the inset gel in Fig. 1). This result has also shown that human β_2m is suitable for formation of a stable complex with Mamu-A*02 heavy chain. A single crystal (Fig. 2) was obtained in 6–8 weeks under optimized conditions. The Mamu-A*02 complex crystal belongs to space group $C2$, with unit-cell parameters a = 124.11, b = 110.45, c = 100.06 Å, and contains two molecules (45 kDa each) in the asymmetric unit. Selected data statistics are shown in Table 1. The electron-density map (Fig. 3) further demonstrated the presence of the complete Mamu-A*02–β_2m–GY9 complex in the crystal. The crystal structure has been successfully determined by molecular replacement using the full sequence of the structure of Mamu-A*01 complex (PDB code 1zln; Chu, Lou, Wai *et al.*, 2006) as a search model. The availability of the structure being resolved by molecular replacement will greatly benefit our understanding of rhesus macaque MHC I (Mamu-A*02) presenting pathogenic SIV peptides.

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