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Complex assembly, crystallization and preliminary X-ray crystallographic studies of rhesus macaque MHC Mamu-A*01 complexed with an immunodominant SIV-Gag nonapeptide

Fuliang Chu,[a,b] Zhiyong Lou,[c] Bin Gao,[d] John I. Bell,[e] Zihe Rao[c] and George F. Gao[a]*

[a]Laboratory of Molecular Immunology and Molecular Virology, Center for Molecular Immunology, Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing 100080, People's Republic of China, [b]Graduate School, Chinese Academy of Sciences, Beijing, People's Republic of China, [c]Laboratory of Structural Biology, Tsinghua University, Beijing 100084, People's Republic of China, [d]Infection and Immunity, Institute of Child Health, University College London, London WC1N 1EH, England, and [e]Nuffield Department of Clinical Medicine, Oxford University, Oxford OX3 9DU, England

Correspondence e-mail: gaof@im.ac.cn

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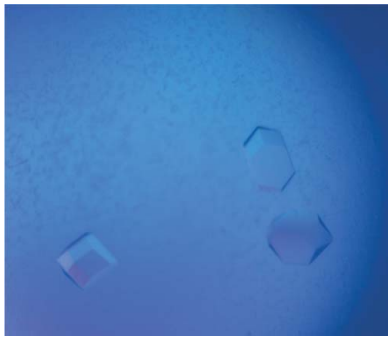

Simian immunodeficiency virus (SIV) infection in rhesus macaques has been used as the best model for the study of human immunodeficiency virus (HIV) infection in humans, especially in the cytotoxic T-lymphocyte (CTL) response. However, the structure of rhesus macaque (or any other monkey model) major histocompatibility complex class I (MHC I) presenting a specific peptide (the ligand for CTL) has not yet been elucidated. Here, using *in vitro* refolding, the preparation of the complex of the rhesus macaque MHC I allele (Mamu-A*01) with human β_2m and an immunodominant peptide, CTPYDINQM (Gag_CM9), derived from SIV Gag protein is reported. The complex (45 kDa) was crystallized; the crystal belongs to space group *I*422, with unit-cell parameters $a = b = 183.8$, $c = 155.2$ Å. The crystal contains two molecules in the asymmetric unit and diffracts X-rays to 2.8 Å resolution. The structure is being solved by molecular replacement and this is the first attempt to determined the crystal structure of a peptide–nonhuman primate MHC complex

1. Introduction

After over two decades of worldwide effort, human immunodeficiency virus (HIV) infection remains a growing threat to human health. At present, scientists are short of any new strategies to directly tackle such a devastating disease. Animal models are thought to play important roles in our understanding of HIV infection for the development of novel therapies or control measures. It has been shown that the nucleotide sequences of the simian immunodeficiency virus (SIV) are closely related to those of HIV-1 and HIV-2 (Chakrabarti *et al.*, 1987; Franchini *et al.*, 1987) and HIV and SIV have similar tropisms for $CD4^+$ T cells (in human and monkey, respectively) and similar pathogenetic processes (Daniel *et al.*, 1985; Klatzmann *et al.*, 1984). This makes SIV infection of monkeys an attractive animal model to test vaccine efficacy *in vivo* and to study HIV pathogenesis in molecular detail (Allen *et al.*, 1998). Despite certain weaknesses, the monkey model has proven to be of enormous value in studying HIV-infection pathogenesis and in evaluating vaccine strategies (Stott & Almond, 1995)

It has been shown that T-cell-mediated immunity, especially the cytotoxic T-cell (CTL) response, is crucial for HIV containment (Desrosiers *et al.*, 1989) and a similar phenomenon has been seen in rhesus macaques with SIV infection (Gotch *et al.*, 1993; Allen *et al.*, 1998). More interestingly, macaque and human major histocompatibility complex class I (MHC I) molecules bind peptides derived from similar regions of the Gag and Env proteins of HIV and SIV (Gotch *et al.*, 1993). Therefore, detailed studies of the rhesus macaque CTL response to SIV infection would definitely benefit our understanding of the process of HIV infection. It has also been shown that some elements of antigen processing as well as the peptide-binding specificities of MHC molecules may have been conserved between humans and rhesus macaques (Allen *et al.*, 1998). However, the structural basis of the binding of rhesus macaque MHC I to its specific peptides has yet to be determined. In this report, we describe the expression of the recombinant rhesus macaque MHC I Mamu-A*01 molecule, its assembly with human β_2-microglobulin (β_2m) and the nanopeptide CTPYDINQM from the Gag region of SIV (Allen *et al.*, 1998) and the crystallization of the whole complex (45 kDa). This Mamu-A*01-restricted peptide has been shown to be important in generating

protective CTL for controlling viral replication and its variants were complicated in SIV escape (Barouch *et al.*, 2005). The crystal structure has been solved and will be published elsewhere. To our knowledge, this is the first attempt to tackle the structure of nonhuman primate class I alleles.

2. Materials and methods

2.1. Preparation of Mamu-A*01 and β_2m proteins as inclusion bodies

The expression vector pET-23c(+)-Mamu-A*01 encoding amino acids 1–276 of the Mamu-A*01 heavy chain was a gift from Professor Norman L. Letvin and Dr Marcelo Kuroda of Harvard Medical School. The plasmid expressing human β_2m, pGMT7-β_2m, was constructed earlier in the laboratory (Willcox *et al.*, 1999). Both recombinant proteins were expressed as inclusion bodies, which were then lysed using a sonicator and centrifuged at 20000*g*. The pellet was 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 X-100 (Zhou *et al.*, 2004).

2.2. Preparation of the Mamu-A*01–β_2m–Gag_CM9 complex

The preparation of the Mamu-A*01–β_2m–Gag_CM9 complex was carried out essentially as previously described by Garboczi *et al.* (1992) with modifications introduced in our laboratory (Zhou *et al.*, 2004). Briefly, the Mamu-A*01 heavy chain and β_2m inclusion bodies were separately dissolved in a solution of 10 m*M* Tris–HCl pH 8.0 and 8 *M* urea. The synthetically prepared SIV_CM9 peptide (CTPYDINQM; SIV Gag amino acids 181–189; this epitope was also called 'p11C, C→M'; Allen *et al.*, 1998) was dissolved in dimethyl sulfoxide (DMSO). Mamu-A*01 heavy chain, β_2m and peptide in a 1:1:3 molar ratio were refolded by the gradual dilution method. After 48 h incubation at 277 K, the remaining soluble portion of the complex was concentrated and then purified by chromatography on a Superdex G-75 (Amersham Biosciences) size-exclusion column followed by Resource-Q (Amersham Biosciences) anion-exchange chromatography.

2.3. Crystallization of the Mamu-A*01–β_2m–Gag_CM9 complex

The purified complex protein (45 kDa) was dialyzed against crystallization buffer (10 m*M* Tris–HCl pH 8.0, 10 m*M* NaCl) and concentrated to 10 mg ml^{-1}. Crystallization trials were set up 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 (10 mg ml^{-1}) and reservoir crystallization buffer was placed over a well containing 200 µl reservoir solution using a VDX plate (HR3-142, Hampton Research). Crystals were obtained in 5–7 d with solution No. 4 of Crystal Screen I [0.1 *M* Tris–HCl pH 8.5, 2.0 *M* ammonium sulfate) and solution No. 23 of Crystal Screen II [1.6 *M* ammonium sulfate, 0.1 *M* MES pH 6.5, 10%(*v*/*v*) dioxane]. The complex crystallized from both of these conditions containing ammonium sulfate. The conditions yielding crystals were further optimized by variation of the precipitant gradient, the protein concentration and the pH of the buffer. Crystals suitable for data collection were grown from the optimized condition 0.1 *M* Tris pH 8.5, 1.6 *M* ammonium sulfate in 7–10 weeks.

2.4. Data collection and processing

Data collection from the Mamu-A*01 complex was performed 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) equipped with 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 2.8 Å. Data were indexed and scaled using *DENZO* and the *HKL*2000 software package (Otwinowski & Minor, 1997).

3. Results and discussion

The complex refolding resulted in yields of approximately 10% of the correctly folded complex (45 kDa), which could be purified to

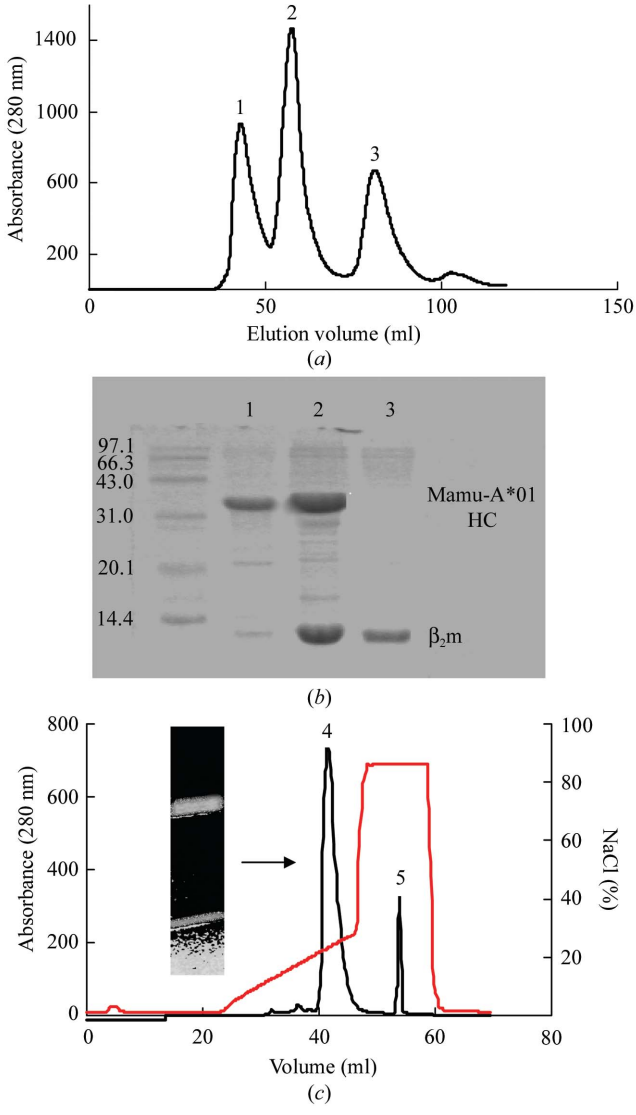

Figure 1
Purification of the refolded complex of Mamu-A*01 heavy chain with human β_2m and SIV Gag_CM9 peptide by FPLC Superdex G75 gel-filtration and Resouce-Q anion-exchange chromatography (Amersham). (*a*) Gel-filtration profile of the refolded products. Peak 1 represents aggregated heavy chain, peak 2 represents the correctly refolded complex (45 kDa) and peak 3 represents the abundant β_2m. (*b*) Reduced SDS–PAGE gel (15%) of the purified proteins. The left column corresponds to molecular-weight markers. The line labelled 1 represents the peak 1, line 2 represents the peak 2 and line 3 represents the peak 3 fractions. (*c*) Results of further purification of the refolded complex (peak 2) by anion exchange. Peak 4 represents the Mamu-A*01 complex, which was eluted at an NaCl concentration of 14–25 m*M*. Peak 5 represents the different charged complex eluted using a higher concentration of salt. Inset: reduced SDS–PAGE gel (15%) for peak 4.

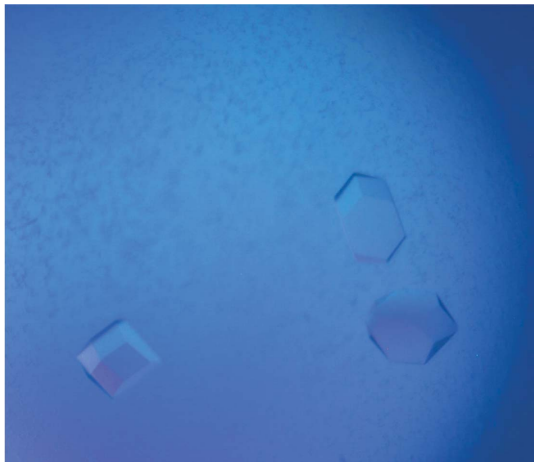

Figure 2
Typical crystals of the Mamu-A*01–β_2m–SIV Gag_CM9 peptide complex.

Table 1
X-ray diffraction data and processing statistics.

Unit-cell parameters (Å, °)	$a = b = 183.8$, $c = 155.2$, $\alpha = \beta = \gamma = 90°$
Space group	$I422$
Resolution (Å)	50.0–2.8
No. of reflections	62116
Unique reflections	28622
Completeness (%)	87.1 (75.6)
Average $I/\sigma(I)$	5.5 (1.1)
R_{sym}	12.9 (56.3)
Redundancy	2.5 (1.7)

homogeneity by Superdex G-75 size-exclusion chromatography and Resource Q (Amersham Biosciences) anion-exchange chromatography (Fig. 1). The chromatographic profile showed three peaks corresponding to the refolded complex (45 kDa; peak 2), uncomplexed β_2m (peak 3) and non-native aggregated products (peak 1; Figs. 1*a* and 1*b*). The refolded complex was further purified by Resource-Q chromatography and the complex was eluted at an NaCl concentration of 14–25 m*M* (Fig. 1*c*). This result has also confirmed that human β_2m is suitable for formation of a stable complex with rhesus macaque class I MHC heavy chain. Large single crystals (Fig. 2) appeared in 7–10 weeks under optimized conditions. The Mamu-A*01 complex crystals belong to space group $I422$, with unit-cell parameters $a = b = 183.8$, $c = 155.2$ Å. The crystal was estimated to contain two molecules (45 kDa each) in the asymmetric unit and the Matthews coefficient value V_M is about 3.8 Å^3 Da^{-1}, with the solvent content calculated to be about 67.6%. Selected data statistics are shown in Table 1. The electron-density maps confirmed the presence of the whole Mamu-A*01–β_2m–Gag_CM9 complex in the crystals described here. The crystal structure has been successfully determined by molecular replacement using the full sequence of the structure of a human HLA-B*5301 complex (PDB code 1a1m; Smith *et al.*, 1996) as a search model. The detailed structure will be reported in the near future.

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