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## Complex assembly, crystallization and preliminary X-ray crystallographic analysis of the chicken MHC class I molecule BF2\*1501

The chicken major histocompatibility complex (MHC) class I molecules named BF are strongly associated with Marek's disease (MD). A single structure, that of chicken BF2\*2101 from the B21 haplotype, which might provide resistance to MD, has been determined. However, little is known about other structures apart from BF2\*2101. In order to provide further structures of chicken MHC class I molecules, BF2\*1501 and chicken  $\beta_2$ -microglobulin complexed with a nonapeptide (MDV-MEQ<sub>RRR9</sub>) derived from Marek's disease virus MEQ protein (MDV *EcoRI* Q fragment, residues 72–80) were assembled and crystallized. Diffraction data from the crystal were collected to 2.6 Å resolution; the crystal belonged to space group  $P3_121$ , with unit-cell parameters  $a = 125.1$ ,  $b = 125.1$ ,  $c = 80.9$  Å and two molecules in the asymmetric unit. The Matthews coefficient  $V_M$  was  $2.08 \text{ \AA}^3 \text{ Da}^{-1}$ , with a calculated solvent content of 40.78%. These data will be helpful in obtaining insight into the structural basis of the involvement of BF2\*1501 in MD.

### 1. Introduction

Major histocompatibility complex (MHC) class I molecules are membrane-surface glycoproteins that play a crucial role in the process of eliminating intracellular pathogens such as viruses and bacteria (Neeffjes *et al.*, 2011). The ternary peptide–MHC I– $\beta_2$ -microglobulin ( $\beta_2m$ ) complexes (pMHC I) are recognized by specific T-cell receptors (TCRs), with the help of the CD8 molecule on the surface of infected cells. The specific recognition is able to activate cytotoxic lymphocytes (CTL) and then lyse the infected cells (Garboczi *et al.*, 1996). The MHC class I molecule comprises two chains: an  $\alpha$  chain, whose extracellular portion is divided into three domains,  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 3$ , and a light chain,  $\beta_2m$  (Bjorkman *et al.*, 1987).

Chicken major histocompatibility complex (chMHC) is also designated as a B locus, an erythrocyte antigen (Plachy *et al.*, 1992). The B locus, the region that corresponds to the classical MHC in chickens, is much smaller and simpler than the typical mammalian MHC in terms of its genomic organization. Detailed analysis has revealed that the B locus is located on chromosome 16 and is composed of tightly linked polymorphic regions: BF (class I), BL (class II $\beta$ ) and BG (Kaufman *et al.*, 1999). BF includes two genes, *BF1*, which is less expressed, and *BF2*, which is dominantly expressed in antigen-presenting cells. *BF2* has been reported to be related to certain infectious pathogens. Peptide motifs for chMHC I of B21, B15 and B4 haplotypes have been detected by peptide pooling (Wallny *et al.*, 2006). Moreover, the structure of the BF2\*2101 from the B21 haplotype has been determined (Koch *et al.*, 2007). The structure of BF2\*2101 showed that a large central cavity in the peptide-binding groove might contribute to the resistance of the B21 haplotype in Marek's disease (MD). In contrast to BF2\*2101, BF2\*1501 from the B15 haplotype is susceptible to Marek's disease virus (MDV; Dalgaard *et al.*, 2003). Therefore, determination of the structure of BF2\*1501 may illustrate the sensitivity to MD.

MDV is a potent oncogenic herpesvirus which can elicit an acute lymphoproliferative disease within a few weeks and eventually leads to mass mortality of chickens. According to viral genome sequence and organization, MDV is classified as a member of the alpha-herpesvirus subfamily (Ross, 1999). The MDV *EcoRI* Q fragment (MEQ) has been shown to be one of the most important proteins that



can induce tumours (Liu *et al.*, 1997). Since MDV is a highly cell-associated virus, T-cell-mediated response is more important than antibody/B-cell-mediated immune response (Parvizi *et al.*, 2010). Here, we report the expression, refolding, purification and crystallization of chicken BF2\*1501 with a nonapeptide (MDV-MEQ<sub>RRR9</sub>) from the MDV MEQ (MDV *EcoRI* Q fragment, residues 72–80) protein.

## 2. Materials and methods

### 2.1. Expression of BF2\*1501 and chicken $\beta_2m$ proteins

BF2\*1501 heavy-chain cDNA (GenBank accession No. L28958, residues 22–291) and chicken  $\beta_2m$  (GenBank accession No. BAD22696, residues 22–119) were amplified by RT-PCR from a specific pathogen-free (SPF) chicken. The BF2\*1501 and chicken  $\beta_2m$  genes were ligated into the pET21a expression vector (Novagen) and transformed into *Escherichia coli* strain BL21 (DE3). The recombinant proteins were both expressed as inclusion bodies. When the bacteria reached an OD<sub>600</sub> of about 0.6, isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM to induce protein expression. After 5 h, the cells were harvested by centrifugation at 6000g for 10 min. After lysis by sonication, the cells were centrifuged at 16 000g and the pellets were washed three times with buffer (20 mM Tris–HCl pH 8.0, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.5% Triton X-100). Finally, BF2\*1501 heavy-chain and  $\beta_2m$  inclusion bodies were dissolved in guanidinium chloride (Gua–HCl) buffer [6 M Gua–HCl, 50 mM Tris–HCl pH 8.0, 10 mM EDTA, 100 mM NaCl, 10% (v/v) glycerine, 10 mM DTT] to a concentration of 30 mg ml<sup>-1</sup> (Chen *et al.*, 2011).

### 2.2. Refolding and purification of the BF2\*1501 complex

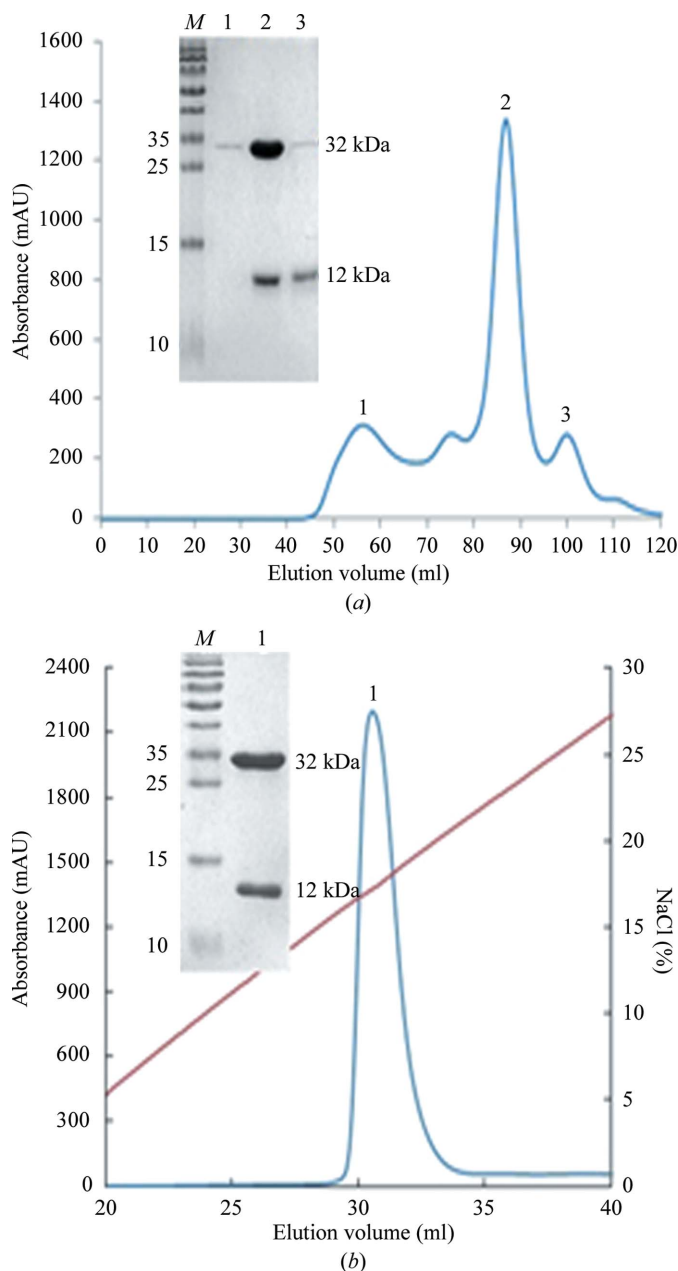
The protocol was essentially the same as described previously (Garboczi *et al.*, 1992) with modification of the refolding temperature from 283 to 277 K, the refolding buffer volume from 200 to 500 ml and the molar ratio of heavy chain to light chain from 1:2 to 1:1 (Pan *et al.*, 2011). The whole refolding process took about 24 h. 1 ml dissolved  $\beta_2m$  inclusion bodies was first added gradually to 500 ml refolding buffer (100 mM Tris–HCl pH 8.0, 2 mM EDTA, 400 mM L-Arg, 0.5 mM oxidized glutathione, 5 mM reduced glutathione) and incubated at 277 K. After 8 h, 5 mg nonapeptide (RRRRKQTDY) dissolved in dimethyl sulfoxide (DMSO) was added to the solution. Half an hour later, 3 ml dissolved BF2\*1501 heavy-chain inclusion bodies were added gradually into the solution and incubated at 277 K for a further 8 h. After refolding, the soluble portion was concentrated and purified by chromatography on a Superdex 200 16/60 HiLoad (GE Healthcare) size-exclusion column followed by Resource Q (GE Healthcare) anion-exchange chromatography.

### 2.3. Crystallization of the BF2\*1501 complex

The purified protein was dialysed against crystallization buffer (20 mM Tris–HCl pH 8.0, 50 mM NaCl) and concentrated to 6 and 12 mg ml<sup>-1</sup>. The commercial kits Index, Crystal Screen, Crystal Screen 2 (Hampton Research, California, USA) consisting of pre-formulated solutions were used for crystallization. Two drops with 1  $\mu$ l protein solution (6 and 12 mg ml<sup>-1</sup>) and 1  $\mu$ l reservoir crystallization buffer were placed over a well containing 160  $\mu$ l reservoir solution using a VDX plate (Hampton Research) at 291 K. 10 d later, crystals were obtained using Index solution No. 6 (0.1 M Tris–HCl pH 8.5, 2.0 M ammonium sulfate) at 291 K.

### 2.4. Data collection and processing

Diffraction data were collected on beamline BL17U at the Shanghai Synchrotron Radiation Facility (SSRF; Shanghai, People's Republic of China) at a wavelength of 0.97916 Å using an ADSC 315 imaging-plate detector. Before diffraction, the crystal was first immersed in reservoir solution containing 20% (v/v) glycerol as cryoprotectant for a few seconds and then flash-cooled in liquid



**Figure 1** Purification of the refolded BF2\*1501 complex (BF2\*1501, chicken  $\beta_2m$  and the epitope MDV-MEQ<sub>RRR9</sub>) by FPLC Superdex 200 16/60 HiLoad gel-filtration and Resource-Q anion-exchange chromatography (GE Healthcare). (a) Gel-filtration profile of the refolded products. Peak 1 corresponds to the aggregated BF2\*1501 heavy chain, peak 2 corresponds to the correctly refolded complex (~44 kDa) and peak 3 corresponds to excess chicken  $\beta_2m$ . Inset, reduced SDS-PAGE gel (15%) for peaks 1, 2 and 3. Lane M contains molecular-mass markers (labelled in kDa). (b) Anion-exchange chromatography profile of the refolded products. Peak 1 corresponds to correctly refolded complex, which was eluted at an NaCl concentration of 16–20%. Inset, reduced SDS-PAGE gel (15%) for peak 1. Lane M contains molecular-mass markers (labelled in kDa).

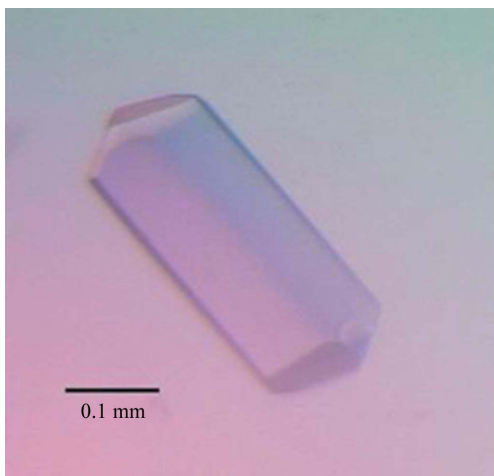
nitrogen (Zhang *et al.*, 2011). The collected intensities were indexed, integrated, corrected for absorption, scaled and merged using *HKL-2000* (Otwinowski & Minor, 1997).

## 2.5. Comparison of BF2\*1501 with other class I molecules

The amino-acid sequence of BF2\*1501 was aligned with those of typical BF2\*2101, human, mouse, bovine, horse and swine MHC class I molecules using *DNAMAN* v.5.2.2 (Raiola *et al.*, 2004). The GenBank accession numbers are as follows: BF2\*2101, AY234769; BoLA-N\*01301, CAA56909; ELA\_7-6, AY225155; SLA-1\*1502, HQ909439; H-2Kb, V00746; HLA-A2, U56825.

## 3. Results and discussion

In each case, about a 10% yield of the stable complex was obtained by refolding *in vitro*. The refolded proteins were first purified by Superdex 200 16/60 HiLoad size-exclusion chromatography (GE Healthcare; Fig. 1*a*). The profile revealed three peaks, which were aggregated BF2\*1501 heavy chain (peak 1), the correctly refolded



**Figure 2**  
Photograph of the crystal used for diffraction analysis.

**Table 1**

X-ray diffraction data and processing statistics.

Values in parentheses are for the highest resolution shell.

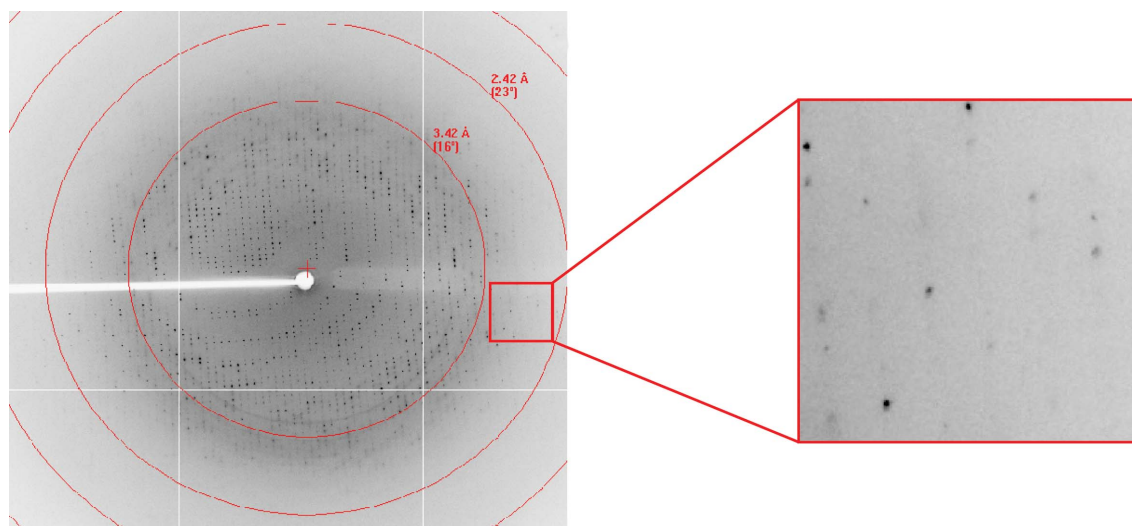
Space group	<i>P</i> 3 <sub>1</sub> 21
Unit-cell parameters (Å)	<i>a</i> = 125.1, <i>b</i> = 125.1, <i>c</i> = 80.9
Volume of the unit cell (Å <sup>3</sup> )	1096701.6
Resolution range (Å)	50.00–2.60 (2.60–2.69)
Total No. of reflections	205783
No. of unique reflections	24077
Completeness (%)	100.0 (100.0)
Average <i>I</i> /σ( <i>I</i> )	22.119 (5.194)
<i>R</i> <sub>merge</sub> † (%)	11.7 (54.0)
Average multiplicity	9.0 (9.1)

†  $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$ , where  $I_i(hkl)$  is the observed intensity and  $\langle I(hkl) \rangle$  is the average intensity from multiple measurements.

complex (peak 2, ~44 kDa) and excess β<sub>2m</sub> (peak 3). The collected complex was further purified by Resource Q anion-exchange chromatography. The specific peak appeared at a NaCl concentration of 16–20% (Fig. 1*b*). SDS-PAGE analysis showed two bands corresponding to the expressed molecular weights of BF2\*1501 (~32 kDa) and chicken β<sub>2m</sub> (~12 kDa) (see inset in Fig. 1).

After purification and concentration, the BF2\*1501–MDV-MEQ<sub>RRR9</sub> complex was set up for crystallization screening. Many single crystals appeared using Index solution No. 6 (0.1 *M* Tris–HCl pH 8.5, 2.0 *M* ammonium sulfate) at 291 K after 10 d. After optimization, a single crystal was used for diffraction (Fig. 2) that grew from a solution consisting of 0.1 *M* Tris–HCl pH 8.5, 1.9 *M* ammonium sulfate at 291 K after 10 d. The crystal belonged to space group *P*3<sub>1</sub>21, with unit-cell parameters *a* = 125.1, *b* = 125.1, *c* = 80.9 Å, and diffracted to 2.6 Å resolution (Fig. 3, Table 1). The Matthews coefficient value  $V_M$  was 2.08 Å<sup>3</sup> Da<sup>−1</sup>, with a calculated solvent content of 40.78%.

A multiple amino-acid sequence alignment of BF2\*1501 with other known MHC class I molecules was performed. A deletion of five amino acids was a notable difference between chicken MHC class I molecules and those from other species. Otherwise, chicken BF2 molecules share very low homology with those from other species. BF2\*1501 has 42, 42.8, 42.8, 43.9 and 43.9% sequence identity to the swine, bovine, equine, mouse and human class I molecules, respectively. However, different haplotypes in chicken share high homology



**Figure 3**  
Diffraction pattern of the chicken MHC I molecule. The high-resolution diffraction spots are highlighted in the box.

with each other. BF2\*1501 has 90.7% sequence identity to BF2\*2101. The results suggest that there is an obvious species difference between chicken MHC class I molecules and their bovine, equine, mouse and human homologues.

Structure determination of BF2\*1501 will be useful for epitope vaccine design for Marek's disease and other pathogens. Structure determination and refinement are currently under way.

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