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Purification, crystallization and preliminary X-ray crystallographic studies of swine MHC class I complexed with an FMDV CTL epitope Hu64

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ABSTRACT

Up to now, no crystal structure of swine leukocyte antigen 2 (SLA-2) molecules was reported. In order to elucidate the structure of SLA-2 and to study the cytotoxic T lymphocyte (CTL) epitopes derived from foot-and-mouth disease virus (FMDV), a complex of swine major histocompatibility complex (MHC) class I molecule (SLA-2 haplotype, Hebao allele) with swine β_2-microglobulin and the CTL epitope FMDV-Hu64 (ALLRTATYY) derived from O serotype of FMDV VP1 protein (residues 64–72) was refolded and crystallized. The crystal, which belonged to space group $P2_12_12_1$, diffracted to 2.5 Å resolution and had unit cell parameters a = 48.37, b = 97.75, c = 166.163 Å. These results will help to determine the first structure of a SLA-2 molecule in the context of an FMDV CTL epitope.

1. Introduction

Major histocompatibility complex (MHC) class I molecules are glycoproteins which play a crucial role in generating specific cellular immunity against viruses and other intracellular pathogens (Wang et al., 2015). Endogenous antigens are degraded into short peptides by the cellular proteasome. The peptides with 8–10 amino acid residues are transported by transporter associated with antigen processing (TAP) into endoplasmic reticulum (ER), where the heavy chain of MHC class I molecules are assembled with an optimal peptide and the light chain of β_2 microglobulin (β_2m). The peptide is just located in the groove comprised of α1 and α2 domains of the MHC class I molecules (Zweerink et al., 1993). The peptide bound to MHC class I is presented onto the surface of the antigen presentation cells to recognize the specific T-cell receptors (TCRs) of the cytotoxic T lymphocytes (CTLs) and the peptide should be called CTL epitope (Klein et al., 1993; Xia et al., 2014). Subsequently, CTLs intrigued by epitopes should secrete cytokines and then kill the target cells(Oelke et al., 2000). In order to further study the antigen presentation for CTL epitopes, analysis of structures of MHC class I is essential. Swine MHC class I gene group, also known as swine leukocyte antigen class I (SLA-I), contains three functional loci: SLA-1, SLA-2 and SLA-3 (Renard et al., 2006). Recently, the crystals of SLA-1 and SLA-3 proteins had been reported (Fan et al., 2016; Zhang et al., 2011), however, no any crystal of SLA-2 was reported. Especially, there are no any FMDV CTL epitopes to be used to crystallize the SLA crystals. In this article, utilizing in vitro refolding and crystallization-condition screening, the SLA-2 molecule was successfully crystallized, making it possible to define the structure of the SLA-2-HB haplotype 01 molecule (SLA-2*HB01) complexed with an FMDV CTL epitope.

2. Materials and methods

2.1. Preparation of SLA-2*HB01 and β_2m proteins

The extracellular domain of the SLA-2 haplotype HB allele 01 coding for 275 amino acids (GenBank accession No. AB602431) was cloned and ligated into cloning vector pMD19-T simple vector as previously described (Gao et al., 2012a). For convenience of cloning, *Nde* I and *Xho* I restriction sites were used in the two terminals of the extracellular domain of the SLA-2*HB01. After digesting the pMD19-T simple vector with *Nde* I and *Xho* I for about 5 h, the target SLA-2*HB01 gene was extracted and inserted into the prokaryotic expression vector pET-21a (+) (Kindly provided by professor George F. Gao, Institute of Microbiology, Chinese Academy of Sciences) (Liu et al., 2012). The pET-21a (+) containing the swine β_2m (sβ_2m) gene had previously

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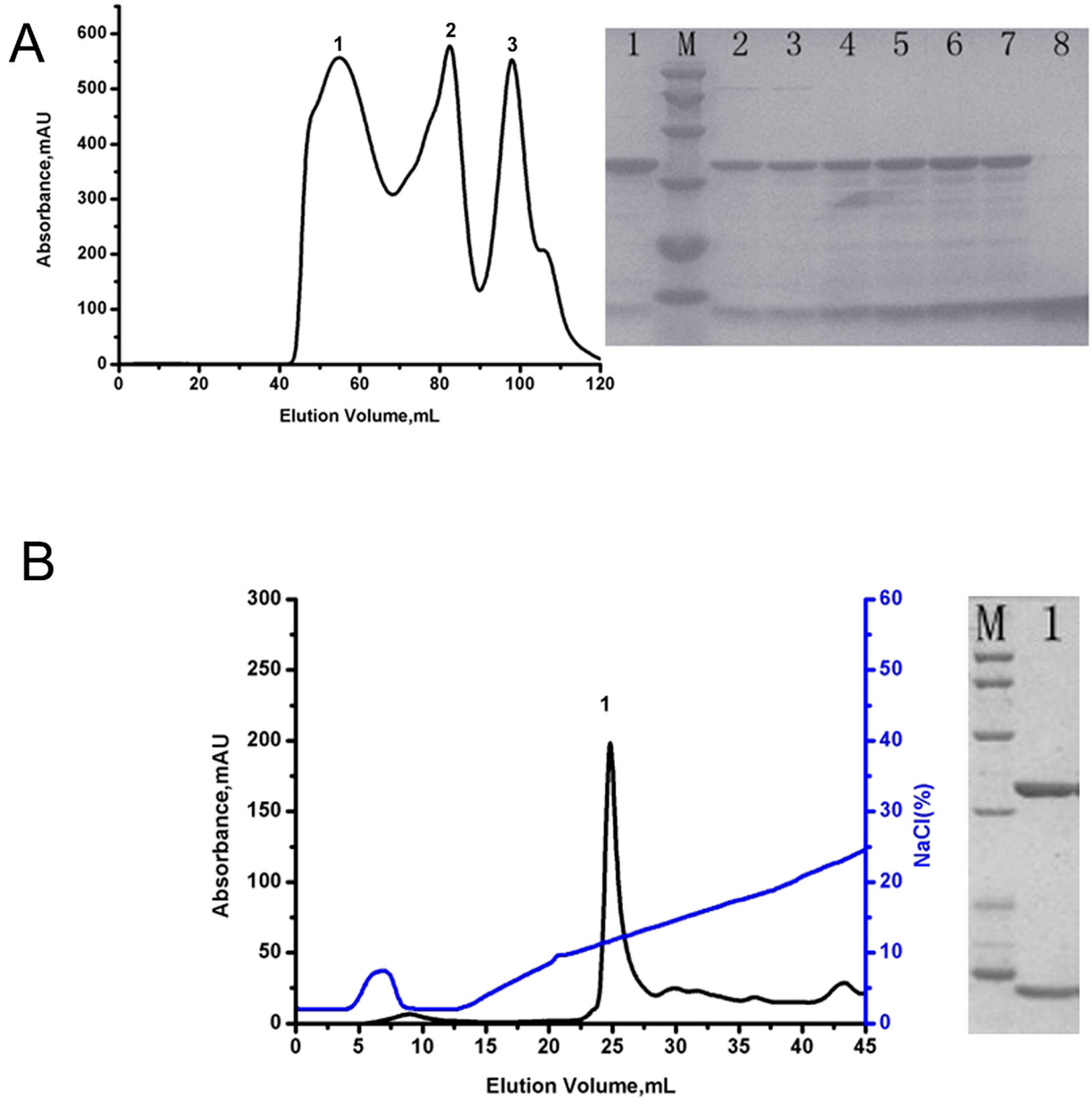

Fig. 1. Purification of the refolded the heavy chain of SLA-I complex (SLA-2*HB01) with sβ_2m and the epitope FMDV-Hu64 by FPLC Superdex 200 16/60 Hi-Load gel-filtration and Resource Q anion-exchange chromatography (GE Healthcare). (A) Gel-filtration profile of the refolded products. Peak 1 contains aggregated heavy chains of SLA-I, peak 2 represent correctly refolded SLA-I complex (~44 kDa) and peak 3 represents sβ_2m. Inset: reduced SDS–PAGE gel (15%) for samples 1, 2-7 and 8 corresponding to peak 1, 2 and 3, respectively. The molecular-weight markers (kDa) was 97.1 kDa, 66.3 kDa, 43.0 kDa, 31.0 kDa, 20.1 kDa and 14.4 kDa. (B) Results of further purification of the refolded products by Q anion-exchange chromatography. Peak 1 represents the SLA-I complex, which was eluted at an NaCl concentration of 12.5–14.8%. Inset: reduced SDS–PAGE gel (15%) for peak 1.

been constructed by our group. The two recombinant plasmids were transformed into *Escherichia coli* strain BL21 (Rosetta) and induced to express the SLA-2*HB01 and sβ_2m proteins. The inclusion bodies were extracted as previously (Zhang et al., 2011). Briefly, the expression strain was inoculated into 2 L Luria–Bertani medium (LB) and shockly incubated at 180 rpm at 37 °C. When the OD_{600} value increased to 0.5, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the incubated cells to a final concentration of 1 mmol/L to induce protein expression. 5 h later, the bacteria were harvested, lysed and centrifugated for 15 min at 6000 rpm at 4 °C. The precipitate was then dissolved in cold phosphate-buffered saline (PBS) containing 1 mmol/L Dithiothreitol (DTT), then sonicated and centrifuged at 16000 rpm for 15 min. The supernatants were removed while the pellets were collected. After being washed for three times by using a solution containing of 10 mmol/L EDTA, 1 mmol/L DTT, 50 mmol/L Tris–HCl, 100 mmol/L NaCl, 0.5% Triton X-100. Finally, the inclusion bodies were dissolved in guanidinium chloride buffer [6 mol/L guanidinium chloride, 50 mmol/L Tris–HCl pH 8.0, 100 mmol/L NaCl, 10 mmol/L EDTA, 10 mmol/L DTT, 100 10%(*v*/v) glycerine] to a protein concentration of 30 mg mL^{-1}.

2.2. Refolding of the SLA-2*HB01-Hu64-sβ_2m

The SLA-2*HB01-Hu64-sβ_2m (SLA-I) complex was prepared essentially as described previously by Garboczi et al., (1992) with modifications introduced by Pan et al., (2011); Xiao et al., (2016). Firstly, the eptitope Hu64 (ALLRTATYY) derived from O serotype of FMDV VP1 protein residues 64–72 was dissolved in dimethyl sulfoxide (DMSO).