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Short communication

Structures and homology modeling of chicken major histocompatibility complex protein class I (BF2 and β2m)

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Abstract

In order to elucidate the two-dimensional (2D) and three-dimensional (3D) structures of chicken major histocompatibility complex (MHC) class I protein (BF2 and β2m)[1] and further reconstruct their complex identifying the virus-derived antigenic peptides, the mature protein of *BF2* and *β2m* genes were expressed solubility in pMAL-p2X/*Escherichia coli*. TB1 system. The expressed MBP-BF2- and MBP-β2m-fusion proteins were purified, and cleaved by the factor Xa protease. Subsequently, the monomers were further separated, and the purified MBP-BF2, -β2m, and MBP were analyzed by circular dichroism (CD) spectrum. The contents of α-helix, β-sheet, turn, and random coil in BF2 protein were 72, 102, 70, and 90 amino acids (aa), respectively. The β2m proteins displayed a typical β-sheet and the contents of α-helix, β-sheet, turn, and random coil were 0, 46, 30, and 22 aa, respectively. Homology modeling of BF2 and β2m proteins were similar as the 3D structure of human MHC class I (HLA-A2). The results showed that pMAL-p2X expression and purification system could be used to obtain the right conformational BF2 and β2m proteins, and the 2D and 3D structures of BF2 and β2m were revealed to be similar to human's. The recombinant BF2 and β2m-based proteins might be a powerful tool for further detecting antigenic peptides.
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Keywords: Chicken; BF2; β2m; Structure; Homology modeling

1. Introduction

Immune response covers a series of physiological and biochemical reactions in all vertebrate to resistant to viral and bacterial infections (Strominger, 2002). The key part of immune system is the production of the associated proteins including major histocompatibility complex (MHC) classes I and II, immunoglobulin, cytokines, complement, and so on (Solheim, 1999). Especially, classical MHC class I is a membrane surface protein, and its mainly function is to bind antigenic peptides, and present it to T-cell receptor (TCR) to trigger the cell immune response (Collins, 2004). Most studies have focused on the three-dimensional crystal structure (3D) of human classical MHC class I protein complex. For example, *HLA-A2* is consisted of a heavy chain (α-chain) and a light chain (β2m), and the peptide-binding domain (PBD) formed by α1 and α2 domains by constructing from two adjacent helical regions on top of an eight-stranded beta-sheets (Bjorkman et al., 1987). Moreover, a detailed examination of the antigen-binding groove confirms that there are six pockets at the bottom of the groove, which are suited for binding side-chains from antigenic peptides (Bjorkman et al., 1987; Madden, 1995; Saper et al., 1991). What catch our eyes was that human's *HLA-A2*, *β2m*, $CD_8\alpha\alpha$ and a HIV peptide had been expressed in *E. coil* and the 3D structure of complex was studied in detail (Gao et al., 1997). Krogsgaard et al. (2005) have also reported that the different biology activations of agonist-peptide and endogenous peptide on the base of constructing the soluble peptide-MHC heterodimers

Abbreviations: aa, Amino acids; AIV, Avian influenza virus; *β2m*, Beta 2-microglobulin; BF2, Chicken major expressed MHC class I; CD, Circular dichroism; HLA-A2, One of the human major expressed MHC class I; MBP, Maltose-binding protein; MHC, Major histocompatibility complex; PBD, Peptide-binding domain

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[1] *Note: BF2* and *β2m* used in this paper in italic font indicate the names of genes, whereas the normal font indicate the names of protein.

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(Krogsgaard et al., 2005). A recombinant MHC class I protein consisting of the antigenic peptide linked to the mouse MHC-I heavy chain and further linked to human β2-microglobulin (*β2m*) was expressed in *E. coli*, and the expressed protein could partially correctly refolded by serological analysis (Sylvester-Hvid et al., 1999). Simultaneity, a single-chain murine MHC class I molecule (*H-2Kd*) in which the first three domains of *H-2Kd* were fused to *β2m* via a 15-amino acid linker had been expressed in baculovirus, and the expressed fusion protein could bind the *H-2Kd*-restricted photoprobe peptide (Godeau et al., 1992). Recently, the method of HLA-A2 binding antigenic peptide system had been established in vitro (Altman et al., 1996), even the bound-peptide sequences could be predicted and identified (Hakenberg et al., 2003; Hammer, 1995; Parker et al., 1995; Zhao et al., 2003).

In livestock, the recombination protein complex consisting of swine MHC class I, *β2m* and CSFV or FMDV-derived peptide representing T-cell epitopes were expressed in *E. coil* and the recombination protein could refold correctly by serology assays (Oleksiewicz et al., 2002). Comparing with human and mammalian, the studies on MHC class I of chicken or birds were still limited to the primary sequences and its partial functions. Thereinto, the chicken MHC class I (*BF2* and *Rfp-Y*) gene sequences were reported, respectively (Briles et al., 1993; Kaufman et al., 1992; Miller et al., 1994). Since then, Kaufman et al. (1999) have reported the genomic structure of Leghorn chicken MHC: there are two loci-encoding class I heavy chains called *BF1* and *BF2* within a 44 kb DNA segment, and the *BF2* locus is dominantly expressed and has the resistance to certain chicken diseases, whereas the *BF1* locus is less expressed and has a lower mRNA transcript (Kaufman et al., 1999; Miller et al., 2004). We have also revealed that five novel *BF2* lineages and one new *β2m* group exist in three Chinese chicken lines (Yan et al., in press). In order to promote the studies on the conformation structures of bird's MHC class I, chicken *BF2* and *β2m* genes were solubly expressed in *E. coli*, and the two-dimensional secondary structures (2D) of expressed fusion BF2 and β2m proteins were analyzed by far UV circular dichroism (CD) spectrum; in addition, the homology modeling were performed to estimate their 3D structures.

2. Materials and methods

2.1. Plasmids, strains, and reagents

E. coli TB1, pMAL-p2X, Rabbit anti-maltose-binding protein (MBP) antiserum, factor Xa, and amylose affinity resin column were obtained from New England BioLabs. Recombination plasmids pGEM-T/*BF2***SH* and pGEM-T/*β2m-SH* were reported in our earlier studies (Yan et al., in press). Restriction enzymes *Eco*RI, *Hin*dIII, T4 DNA ligase, *Ex*Taq DNA polymerase, dNTPs, IPTG, and X-gal were purchased from TakaRa (TaKaRa Biotechnology (Dalian) Co., Ltd.). Polyvinylidene fluoride (PVDF) filter membrance and Amicon Ultra-15 protein centrifugal filter (30 k) were from Millipore Company. DEAE-Sepharose ion exchange chromatography was from Phamacia Company. 4-Chloro-1-naphthol (4CIN) was from Sigma Corporation.

2.2. Construction of the expression plasmids

Two primer pairs P1/P2 and P3/P4, which containing *Eco*RI in forward primers (P1 and P3) and *Hin*dIII in reverse primers (P2 and P4) were designed to amplify the mature peptide genes from recombination plasmids pGEM-T/*BF2***SH* (AB159064) and pGEM-T/*β2m-SH* (AB162661), respectively. The primer sequences, with the restriction sites being underlined, were as follows: P1: 5′-TCAGAATTCGAGCTCCA TACC CTGCGGTAC-3′; P2: 5′-AGTAAGCTTTGAGATGGCGGGGTTGCTCC-3′; P3: 5′-TCAGAATTCG ACCTGACGCCCAAGGTGCAG-3′; P4: 5′-AGTAAGCTTTCAGAACTCGGGATCCCA CTTG-3′. PCR was carried out in a final volume of 50 μL, containing 10 ng T-easy plasmids as templates. The amplifications were firstly performed at 98 °C for 3 min and 72 °C for 1 min followed by adding ExTaq polymerase, then by 30 cycles (94 °C for 1 min, 65 °C for 1 min, 72 °C for 1.5 min). The final extension was at 72 °C for 10 min. The *β2m* gene was amplified by 30 cycles at 94 °C for 1 min, 65 °C for 45 s, 72 °C for 45 s; then, at 72 °C for 10 min. The PCR products about 1 kb and 300 bp fragments, respectively, were isolated and purified using glass milk kit, then cloned into T-easy vectors, and digested with *Eco*RI and *Hin*dIII. Subsequently, the *BF2* and *β2m* genes were cloned into pMAL-p2X expressed vector at the *Eco*RI and *Hin*dIII restriction sites (named p2X-*BF2* and p2X-*β2m*, respectively) and transfected into *E. coli* TB1. The clones were sequenced on both strands by the Shanghai Sangon Biotechnology Company.

2.3. Expression, SDS-PAGE, and Western-blot

Fusion expression of the *BF2* or *β2m* genes was carried out followed the protocols offered by the New England BioLabs Inc. (NEB, 1991–2001) In brief, *E. coli* TB1/p2X-*BF2* and *E. coli* TB1/p2X-*β2m* plasmids were added, respectively, into 250 mL LB culture, then induced by 0.03 mmol/L IPTG. After further growing at 37 °C for 4 h, the cells were collected by centrifugation at 4 °C 4000 × *g* for 10 min. The pellets were resuspended in column buffer and freezed at −70 °C, then sonicated in an ice bath. After centrifugation at 4 °C 9000 × *g* for 20 min, the supernatant and the pellets were analyzed by SDS-PAGE, respectively. The expressed fusion proteins were named MBP-BF2 and MBP-β2m.

The fusion MBP-BF2 and MBP-β2m proteins were electrophoresed in 12% polyacrylamide SDS-PAGE and visualized using Coomassie Brilliant Blue stain as the reference (Xia et al., 2005). For Western-blot analysis, the protein bands were electrotransferred onto PVDF membrane at 100 V for 1 h by Trans-Blot SD. The filter membrane was blocked

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