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Received 29 October 2007

Accepted 25 December 2007

Expression, purification, crystallization and preliminary X-ray diffraction analysis of grass carp β_2 -microglobulin

β_2 -Microglobulin (β_2 m) is an essential subunit of MHC I molecules; it stabilizes the structure of MHC I and plays a pivotal role in coreceptor recognition. To date, structures of β_2 m have been solved for three different mammals: human, mouse and cattle. In order to illuminate the molecular evolutionary origin of β_2 m, an understanding of its structure in lower vertebrates becomes important. Here, grass carp (*Ctenopharyngodon idellus*) β_2 m (*Ctid*- β_2 m) was expressed, purified and crystallized. Diffraction data were collected to a resolution of 2.5 Å. The crystal belongs to space group $P2_12_12_1$, with unit-cell parameters $a = 38.72$, $b = 40.65$, $c = 71.12$ Å. The Matthews coefficient and the solvent content were calculated to be 2.56 Å Da^{-1} and 52.07%, respectively, for one molecule per asymmetric unit. The structure has been solved by molecular replacement using monomeric human β_2 m as a model.

1. Introduction

β_2 -Microglobulin (β_2 m) constitutes the noncovalently bound light chain of MHC I and appears to stabilize the structure of the heavy chain in that its loss can remove alloantigenic sites on MHC (Michaelsson *et al.*, 2001; Lancet *et al.*, 1979; Krangel *et al.*, 1979). The β_2 ms in teleosts, amphibians and mammals are 97, 97 and 99 residues in length, respectively (Hao *et al.*, 2006). The β_2 m gene is comparatively conserved compared with the diversity and polymorphism of the heavy chain of MHC I and the proteins from different species have similar chemical structures. Furthermore, the β_2 ms from various mammalian species have been found to be able to replace one another in the quaternary structure of the MHC I antigens, suggesting that the conservation of the sequence reflects strong evolutionary pressure to conserve a functionally important conformation.

It has been shown that many important mammalian immune molecules are also found in the lower vertebrates and play similar functions in the immune response. Some residues have also been reported to interact with CD8 molecules when CTLs recognize target cells. Therefore, detailed studies of β_2 m structures from lower vertebrates would definitely benefit our understanding of the immune evolution of β_2 m in different species and of the structure of the MHC I complex. However, to date the crystal structure of β_2 m has only been solved for mammals, such as human, mouse and cattle (Trinh *et al.*, 2002; Becker & Reeke, 1985; Achour *et al.*, 1998). Structures of β_2 m from lower vertebrates have not yet been determined. Here, we report the crystallization of grass carp (*Ctenopharyngodon idellus*) β_2 m (*Ctid*- β_2 m) and describe the expression, refolding and purification of the recombinant *Ctid*- β_2 m protein. To our knowledge, this is the first attempt to tackle the structure of lower vertebrate β_2 ms and to facilitate the solution of the structure of their MHC molecules.

2. Materials and methods

2.1. Preparation of *Ctid*- β_2 m as inclusion bodies

The *Ctid*- β_2 m gene was amplified from the plasmid p2X-*Ctid*- β_2 m, which was constructed previously by our research group (Hao *et al.*, 2006), by the polymerase chain reaction (PCR) with a unique *Nde*I recognition site, a stop codon and a unique *Xho*I recognition site. The

products were ligated into pET21a vector (Novagen) and transformed into *Escherichia coli* strain BL21(DE3) (Chu *et al.*, 2005). The recombinant proteins were expressed as inclusion bodies, which were then lysed by sonication and centrifuged at 2000g. The pellet was washed three times with a solution containing 0.5% Triton X-100 and 20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1 mM DTT and once with the same solution without Triton X-100. The inclusion bodies were dissolved overnight in urea buffer [8 M urea, 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 10 mM EDTA, 10% (v/v) glycerine, 10 mM DTT] with about 1 ml urea buffer per 30 mg of protein.

2.2. Preparation of the refolded recombinant protein *Ctid-β₂m*

The preparation of *Ctid-β₂m* was carried out essentially as previously described by Garboczi *et al.* (1992) with modifications in our laboratory (Chu *et al.*, 2005). Briefly, the *Ctid-β₂m* inclusion bodies were separately dissolved in a solution of 10 mM Tris pH 8.0 and 8 M urea. *Ctid-β₂m* was refolded by the gradual dilution method. After 48 h incubation at 277 K, the remaining soluble portion of *Ctid-β₂m* was concentrated and then purified by chromatography on a Superdex 200 (GE Healthcare) size-exclusion column followed by Resource-Q (GE Healthcare) ion-exchange chromatography.

Figure 1
Purification of refolded *Ctid-β₂m* by FPLC Superdex G-200 gel-filtration and Resource-Q anion-exchange chromatography. (a) Gel-filtration profile of the refolded products. Insert, reduced SDS-PAGE gel (15%) of corresponding purified protein. The right column contains molecular-weight markers (labelled in kDa). (b) Results of further purification of the refolded products by anion-exchange chromatography. The peak was eluted at a NaCl concentration of 3.4–30.8%. Insert, reduced SDS-PAGE gel (15%) of the corresponding purified protein.

2.3. Crystallization of *Ctid-β₂m*

The purified *Ctid-β₂m* was dialyzed against crystallization buffer (10 mM Tris-HCl, 10 mM 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. Two drops containing equal volumes of protein solution (at 5 and 10 mg ml⁻¹) and reservoir crystallization buffer (1 μl each) were placed over a well containing 200 μl reservoir solution using a VDX plate (HR3-142, Hampton Research). Crystals were obtained in 3–5 d using solution No. 22 from Crystal Screen II (0.1 M MES pH 6.5, 12% PEG 20 000), as well as other conditions such as solution No. 41 from Crystal Screen I. Conditions that yielded crystals were optimized by varying the precipitant concentration, the protein concentration and the pH of the buffer and by the use of additives (Hampton Research). Crystals suitable for data collection grew in 3–5 d from the optimized conditions 0.1 M MES pH 6.5, 12% PEG 20 000, 3% (v/v) ethanol using 5 mg ml⁻¹ protein concentration.

2.4. Data collection and X-ray diffraction analysis

Data were collected from the *Ctid-β₂m* crystals in-house using a Rigaku MicroMax007 rotating-anode X-ray generator operated at 40 kV and 20 mA (Cu Kα; λ = 1.5418 Å) equipped with an R-Axis VII⁺⁺ image-plate detector. The crystals were soaked for several minutes in reservoir solution supplemented with 20% glycerol as a cryoprotectant and then flash-cooled directly in liquid nitrogen. A complete data set was collected to 2.5 Å. Data were indexed and scaled using DENZO and the HKL-2000 software package (Otwinowski & Minor, 1997).

Figure 2
Typical crystals of *Ctid-β₂m*.

Table 1

X-ray diffraction data and processing statistics.

Space group	$P2_12_12_1$
Unit-cell parameters ($\text{\AA}$, $^\circ$)	$a = 38.72$, $b = 40.65$, $c = 71.12$, $\alpha = \beta = \gamma = 90$
Total no. of reflections	28114
Unique reflections	4156
Completeness (%)	98.5 (96.1)
$R_{\text{merge}}^\dagger$ (%)	7.2 (20.0)
$I/\sigma(I)$	15.5 (7.0)
Multiplicity	6.76 (6.96)

$^\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \overline{I(hkl)}| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the i th observation of reflection hkl and $\overline{I(hkl)}$ is its mean intensity.

3. Results and discussion

Refolding of *Ctid*- β_2 m resulted in an approximately 15% yield of *Ctid*- β_2 m inclusion bodies, which could be purified to homogeneity by Superdex G-200 size-exclusion chromatography and Resource-Q anion-exchange chromatography (Fig. 1). The chromatography profile showed a single peak corresponding to the expected monomeric *Ctid*- β_2 m protein (10.7 kDa; Fig. 1a). The refolded *Ctid*- β_2 m was further purified by Resource-Q chromatography and eluted at a NaCl concentration of 3.4–30.8% (Fig. 1b). Ideal single crystals (Fig. 2) appeared in 3–5 d under optimized conditions. The *Ctid*- β_2 m crystals belong to space group $P2_12_12_1$, with unit-cell parameters $a = 38.72$, $b = 40.65$, $c = 71.12$ $\text{\AA}$. Selected data statistics are shown in Table 1. The electron-density map confirmed the presence of the whole *Ctid*- β_2 m molecule in the crystals. The crystal structure has

successfully been determined by molecular replacement using the full sequence of the structure of human β_2 m (PDB code 1lds) as a search probe. The detailed structure will be published in the near future.

This work was completed in the laboratory of Professor George F. Gao at the Institute of Microbiology, Chinese Academy of Sciences (IMCAS) and was supported by a grant from the National Key Basic Research Program of China (973 Program, 2007CB815805).

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