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

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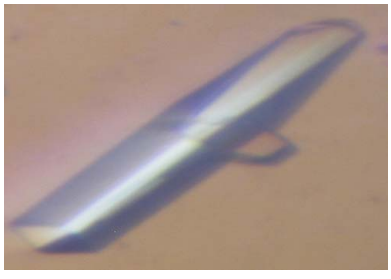

β_2-Microglobulin (β_2m) is an essential subunit of MHC I molecules; it stabilizes the structure of MHC I and plays a pivotal role in coreceptor recognition. To date, structures of β_2m have been solved for three different mammals: human, mouse and cattle. In order to illuminate the molecular evolutionary origin of β2m, an understanding of its structure in lower vertebrates becomes important. Here, grass carp (*Ctenopharyngodon idellus*) β_2m (*Ctid*-β_2m) 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_2m as a model.

1. Introduction

β_2-Microglobulin (β_2m) 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 $\beta_2$ms in teleosts, amphibians and mammals are 97, 97 and 99 residues in length, respectively (Hao *et al.*, 2006). The β_2m 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 $\beta_2$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_2m structures from lower vertebrates would definitely benefit our understanding of the immune evolution of β_2m in different species and of the structure of the MHC I complex. However, to date the crystal structure of β_2m 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_2m from lower vertebrates have not yet been determined. Here, we report the crystallization of grass carp (*Ctenopharyngodon idellus*) β_2m (*Ctid*-β_2m) and describe the expression, refolding and purification of the recombinant *Ctid*-β2m protein. To our knowledge, this is the first attempt to tackle the structure of lower vertebrate $\beta_2$ms and to facilitate the solution of the structure of their MHC molecules.

2. Materials and methods

2.1. Preparation of *Ctid*-β_2m as inclusion bodies

The *Ctid*-β_2m gene was amplified from the plasmid p2X-*Ctid*-β_2m, 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 2000*g*. The pellet was washed three times with a solution containing 0.5% Triton X-100 and 20 m*M* Tris–HCl, 100 m*M* NaCl, 1 m*M* EDTA, 1 m*M* DTT and once with the same solution without Triton X-100. The inclusion bodies were dissolved overnight in urea buffer [8 *M* urea, 50 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl, 10 m*M* EDTA, 10%(*v*/*v*) glycerine, 10 m*M* DTT] with about 1 ml urea buffer per 30 mg of protein.

2.2. Preparation of the refolded recombinant protein *Ctid*-β_2m

The preparation of *Ctid*-β_2m was carried out essentially as previously described by Garboczi *et al.* (1992) with modifications in our laboratory (Chu *et al.*, 2005). Briefly, the *Ctid*-β_2m inclusion bodies were separately dissolved in a solution of 10 m*M* Tris pH 8.0 and 8 *M* urea. *Ctid*-β_2m was refolded by the gradual dilution method. After 48 h incubation at 277 K, the remaining soluble portion of *Ctid*-β_2m 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.

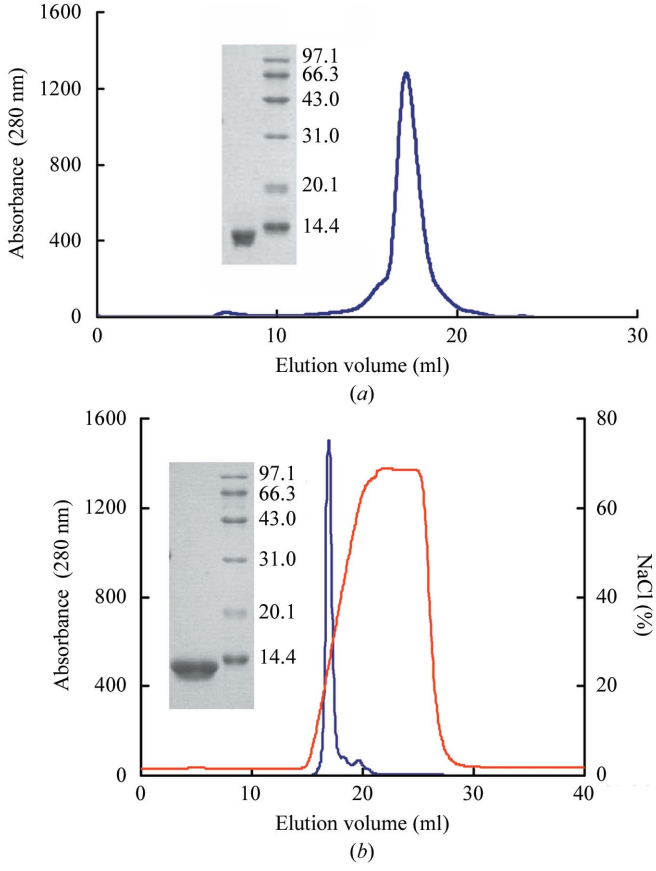

Figure 1
Purification of refolded *Ctid*-β_2m by FPLC Superdex G-200 gel-filtration and Resource-Q anion-exchange chromatography. (*a*) Gel-filtration profile of the refolded products. Insert, reduced SDS–PAGE gel (15%) of corresponding purified protein. The right column contains molecular-weight markers (labelled in kDa). (*b*) Results of further purification of the refolded products by anion-exchange chromatography. The peak was eluted at an NaCl concentration of 3.4–30.8%. Insert, reduced SDS–PAGE gel (15%) of the corresponding purified protein.

2.3. Crystallization of *Ctid*-β_2m

The purified *Ctid*-β_2m was dialyzed against crystallization buffer (10 m*M* Tris–HCl, 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. 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 $K\alpha$; 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).

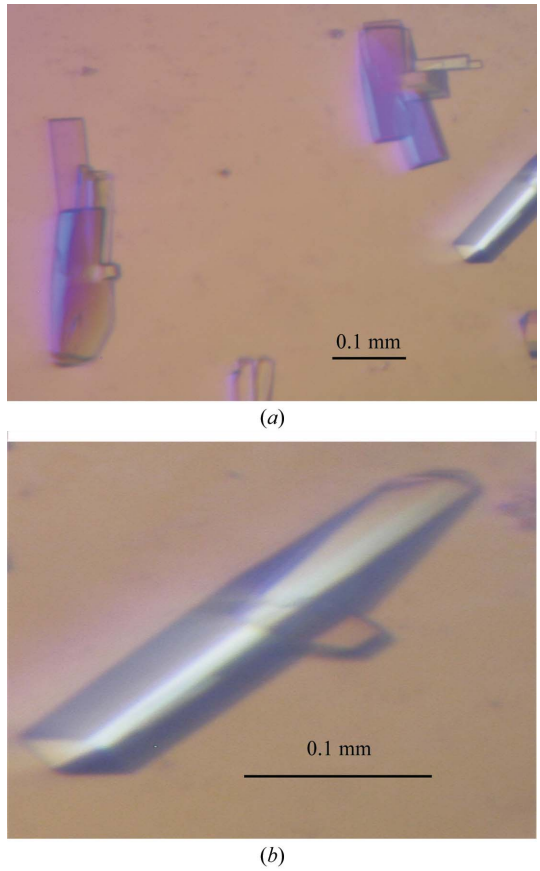

Figure 2
Typical crystals of *Ctid*-β_2m.

Table 1
X-ray diffraction data and processing statistics.

Space group	$P2_12_12_1$
Unit-cell parameters (Å, °)	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)
$R_{\rm merge}$† (%)	7.2 (20.0)
$I/\sigma(I)$	15.5 (7.0)
Multiplicity	6.76 (6.96)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \overline{I(hkl)}|/\sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\overline{I(hkl)}$ is its mean intensity.

3. Results and discussion

Refolding of *Ctid*-β_2m resulted in an approximately 15% yield of *Ctid*-β2m 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_2m protein (10.7 kDa; Fig. 1*a*). The refolded *Ctid*-β_2m was further purified by Resource-Q chromatography and eluted at a NaCl concentration of 3.4–30.8% (Fig. 1*b*). Ideal single crystals (Fig. 2) appeared in 3–5 d under optimized conditions. The *Ctid*-β_2m crystals belong to space group $P2_12_12_1$, with unit-cell parameters a = 38.72, b = 40.65, c = 71.12 Å. Selected data statistics are shown in Table 1. The electron-density map confirmed the presence of the whole *Ctid*-β_2m molecule in the crystals. The crystal structure has successfully been determined by molecular replacement using the full sequence of the structure of human β_2m (PDB code 1lds) as a search probe. The detailed structure will be published in the near future.

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