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Preliminary crystallographic characterization of the human β2 microglobulin His31Tyr mutant in a tetrameric assembly

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Patients receiving prolonged haemodialysis treatment are exposed to a variety of arthropathies and bone lesions arising from deposition of amyloid material in the skeletal system. β2 microglobulin is the 11.7 kDa light chain of the class I major histocompatibility complex, from which it is normally released to plasmatic fluids, transported to kidneys and excreted. Owing to renal failure it accumulates, giving rise to dialysis-related amyloidosis, a severe disease found in patients receiving dialysis for several years. The three-dimensional structure of β2 microglobulin is known to be based on a seven-stranded β-sandwich fold, typical of the class C immunoglobulin superfamily. Analysis of the protein fold in different mutants and/or crystal environments and of its structural stability may help in understanding the molecular bases of amyloid fibril formation and of diseases related to protein misfolding. Here, the preliminary crystallographic analysis of the His31Tyr β2 microglobulin mutant, designed to abolish the copper-ion binding observed in the wild-type protein, is presented. The protein mutant displays increased fold stability, faster folding kinetics and crystallizes in the tetragonal $C222_1$ space group, with unit-cell parameters a = 105.2, b = 150.2, c = 93.7 Å and four molecules per asymmetric unit.

1. Introduction

β2 microglobulin (β2m) is the non-covalently bound light chain of the human class I major histocompatibility complex. The protein (MW $\simeq$ 11.7 kDa) is structured in a β-sandwich domain composed of two facing β-sheets (four stranded and three stranded), typical of the C-type immunoglobulin superfamily (Trinth *et al.*, 2002). As also previously shown by the crystal structure of the whole MHC class I protein (MHC-I) complex (Bjorkman *et al.*, 1987), β2m is stabilized by an intramolecular disulfide bridge connecting Cys25 and Cys80 in the facing β-sheets. *In vivo*, MHC-I continuously exposes β2m on the cell surface, where it may be collected by plasmatic fluids, transported to the kidneys, degraded and excreted under normal conditions. Conversely, in patients receiving haemodialysis for long periods, the plasma concentration of β2m may increase by up to 60-fold (Floege & Ehlerding, 1996). Under such pathological conditions, β2m is responsible for the onset of a human disorder known as haemodialysis-related amyloidosis (DRA; Gejyo *et al.*, 1985), in which large amounts of β2m fibrillar aggregates are deposited mainly in the joints and bones, causing a variety of arthropathies and pathological fractures.

As shown by the analysis of amyloid deposits related to some 20 different human diseases (Westermark *et al.*, 2002), the sequences and structures of the proteins responsible for amyloid fibril formation may be totally unrelated. Nevertheless, the deposited fibrils appear to share a common overall architecture (the 'cross-β structure') based on a superhelical arrangement of β-strands paired in a direction orthogonal to the fibril elongation axis (Sunde *et al.*, 1997; Serpell *et al.*, 1999). Understanding the molecular mechanisms of β2m stability *versus* its propensity to misfolding, potentially leading to higher molecular aggregates and eventually to fibril formation, is a fundamental step for the understanding of amyloid-based diseases such as DRA. In this context, we undertook the expression, purification and preliminary crystallographic analysis of the His31Tyr single-site β2m mutant (H31Yβ2m). In fact, it has recently been shown that His31 is the main residue responsible for copper binding in wild-type β2m. Moreover, H31Y β2m has recently shown to display a higher stability of the correctly folded form and a loss of copper-ion binding capability relative to the wild-type species (De Lorenzi *et al.*, 2002; Eakin *et al.*, 2002).

2. Materials and methods

2.1. Mutagenesis, expression and purification

Site-specific mutation of β2m cDNA, cloned in the pHN1 plasmid (a generous gift from Dr

J. Bell, Oxford, England), was performed using the QuikChange Site-Directed Mutagenesis Kit supplied by Stratagene (La Jolla, CA, USA). The primers for the mutated plasmid preparation were the following: 5′-GC TAT GTG TCT GGG TTT **TAT** CCA TCC GAC ATT GAA G and 3′-C TTC AAT GTC GGA TGG **ATA** AAA CCC AGA CAC ATA GC. *Escherichia coli* strain BL21DE3 was transformed with the mutated plasmid and the protein expression was regulated by the Lac operon promoter. H31Yβ2m was purified as previously described (Esposito *et al.*, 2000); the protein sequence was confirmed by a combination of amino-acid sequence and mass spectrometry, as previously reported (De Lorenzi *et al.*, 2002).

2.2. Crystallization and data collection

Crystallization conditions for recombinant H31Yβ2m were screened according to the sparse-matrix method (Jancarik & Kim, 1991) using commercially available crystallization kits (Hampton Research, Laguna Hills, California, USA). The best H31Yβ2m crystals were obtained using the hanging-drop vapour-diffusion method (McPherson, 1992). The crystallization reservoir solution was composed of 31% PEG 4000, 25% glycerol, 0.2 *M* ammonium acetate, 0.1 *M* sodium acetate pH 5.6. The crystal-growth droplet (3 µl) was composed of 1 µl protein solution (16–19 mg ml^{-1}), 1 µl reservoir solution and 1 µl 0.05 *M* sodium acetate pH 5.6. Addition of 0.1 *M* sodium acetate proved useful in controlling excessive crystal nucleation during growth. All crystallization experiments were performed at 294 K. Single crystals of about 0.07 × 0.07 × 0.05 mm grew in about 20 d (Fig. 1).

X-ray diffraction data were collected at 100 K at the ELETTRA (Trieste, Italy; beamline XRD-1, λ = 0.927 Å) and ESRF (Grenoble, France; beamline ID14-4, λ = 0.939 Å) synchrotron facilities; the crystallization mother liquor proved to be a suitable cryoprotectant without further modification. Both X-ray diffraction data sets were complete at the 99.9% level, showing comparable data-reduction statistics (Table 1). Diffraction data collected at the ESRF extended to slightly higher resolution (to about 2.7 Å). Therefore, the latter data set was processed for subsequent analysis using *MOSFLM* and scaled with the programs *SCALA* and *TRUNCATE* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994) at a maximum resolution of 2.79 Å (Table 1).

Figure 1
A view of the H31Yβ2m orthorhombic crystals; the largest dimensions are about 0.07 × 0.07 × 0.05 mm. The thin prismatic crystals present in the picture displayed poorer X-ray diffraction and were not characterized.

Table 1
Data reduction statistics for H31Yβ2m.

Values in parentheses are for the highest resolution shell.

Beamline	ESRF ID14-4
Wavelength (Å)	0.939
Space group	$C222_1$
Resolution range (Å)	30–2.79 (2.84–2.79)
Completeness (%)	99.9 (99.1)
Observed reflections	219876
Unique reflections	18871
R_{sym} (%)	6.3 (37.1)
$\langle I/\sigma(I)\rangle$	19.8 (2.5)
Unit-cell parameters (Å)	a = 105.20, b = 150.17, c = 93.67

3. Results and discussion

From analysis of the diffraction symmetry and of systematic absences, the H31Yβ2m crystals were assigned to the orthorhombic $C222_1$ space group. Calculation of the crystal-packing parameter (V_M = 4.0 Å^3 Da^{-1}; Matthews, 1968) suggests the presence of at least four H31Yβ2m molecules in the crystal asymmetric unit, corresponding to a solvent content of 69.5%.

Analysis of the self-rotation function, as calculated using the program *AMoRe* (Navaza, 1994), did not provide any evidence of simple rotational symmetry among the expected four protomers contained in the asymmetric unit. Further analysis, *via* molecular replacement using as search model the atomic coordinates of wild-type human β2m (crystallized in a monoclinic form, with one molecule per asymmetric unit; Trinth *et al.*, 2002; PDB code 1lds), allowed solution of the full H31Yβ2m structure. Three of the four H31Yβ2m independent molecules were easily positioned in the asymmetric unit using the program *MOLREP* (Vagin & Teplyakov, 1997) (correlation coefficient and *R* factor of 0.41 and 51.8%, respectively). Rigid-body refinement of the three located molecules was performed using the program *REFMAC*5 (Collaborative Computational Project, Number 4, 1994); accordingly, the *R* factor fell to 41.0%. At this stage, a clear and extended trace of residual electron density became evident in the solvent space contacting the previously located H31Yβ2m molecules. The extra density was interpreted as being indicative of the presence of a fourth mutant protein molecule, which was positioned in the asymmetric unit after solvent-flattening cycles performed with the program *DM* (Cowtan, 1994) (*R* factor 38.7%). The full H31Yβ2m model, composed of four independent protein chains which appear tightly packed, leaving wide solvent channels through the crystal space, is currently under refinement.

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