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Expression, purification, crystallization and crystallographic characterization of the human MHC class I related protein MICA

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Abstract

Crystals of the human MHC-encoded molecule MICA, a homologue of MHC class I proteins, have been grown in hanging-drop vapor-diffusion trials using ammonium sulfate as a precipitating agent with recombinant protein expressed in a baculovirus-based system. Cryo-preserved crystals of MICA belong to the cubic space group $F4_132$ with lattice constants $a = b = c = 260.7$ Å and diffract to a resolution limit of 3.0 Å when cryo-preserved. These crystals do not diffract when handled conventionally.

1. Introduction

The human major histocompatibility complex (MHC) on chromosome 6 harbors a family of HLA class I genes, of which *HLA-A*, *-B* and *-C* encode highly polymorphic and ubiquitously expressed membrane-bound glycoproteins that are non-covalently associated with β_2-microglobulin (β_2m) (Trowsdale, 1993). These heterodimers display intracellularly derived peptides on the cell surface to circulating $CD8^+$ cytotoxic T cells during immune surveillance (Townsend & Bodmer, 1989). In addition to these classical MHC class I genes, there are two genes, *HLA-E* and *-G*, that encode for non-classical MHC class I molecules (Geraghty, 1993). Although the function of HLA-E is unclear, HLA-G seems to play a physiological role at the maternal–fetal interface (Le Bouteiller & Lenfant, 1996).

Recently, a second lineage of MHC class I genes has been identified in the human MHC (Bahram *et al.*, 1994). Members of this family are referred to as *MICA* and *MICB* (MHC class I chain-related genes *A* and *B*). At least one homologue of these genes is conserved in most mammalian species, suggesting that they serve a fundamental function. For *MICA*, 16 alleles, differing at a total of 22 positions in the sequence, have been identified (Fodil *et al.*, 1996). No more than two different amino acids have been observed at any one location in the protein sequence across the 16 alleles. MICA is 337 residues in length. Expression of MICA has so far been found almost exclusively restricted to gastrointestinal epithelium and is presumably stress-induced (Groh *et al.*, 1996). The function of MICA is not known, but the restricted and cell-stress-dependent expression of MICA suggests that this molecule may function as specialized ligand for a subset of T cells in the intestinal epithelium. The protein sequence of MICA shares 20–35% identical amino acids in the $\alpha1$, $\alpha2$ and $\alpha3$ extracellular domains with human and murine MHC class I sequences and thus is, together with *MICB*, the most divergent mammalian MHC class I sequence known (Bahram *et al.*, 1994; Bahram & Spies, 1996). Despite this divergence in sequence, the MICA chain includes most of the amino-acid residues considered critical for the folding of class I chains and thus may have a tertiary structure similar to class I molecules. In contrast to classical MHC class I molecules, MICA is not associated with β_2-microglobulin (β_2m; Groh *et al.*, 1996), a characteristic that it shares with the Zn-α_2-glycoprotein, a soluble MHC class I homologue (Sanchez *et al.*, 1997).

The crystal structures of multiple MHC class I–peptide complexes have produced detailed insights into this versatile receptor–ligand system (Bjorkman *et al.*, 1987; Garrett *et al.*, 1989; Madden *et al.*, 1991). However, several examples illustrate that class-I-related molecules have been adapted for different functions in evolution (Burmeister *et al.*, 1994; Feder *et al.*, 1996; Sanchez *et al.*, 1997). To expand this knowledge, we have initiated an effort to determine the crystal structure of MICA. This will allow us to address how the sequence divergence from class I molecules and the lack of β_2m association affect the structure of this molecule.

2. Material and methods

2.1. *Expression and purification*

Recombinant MICA (allele 001, Fodil *et al.*, 1996) was expressed as a secreted protein in High-Five™ insect cells (Invitrogen) using the BAC-TO-BAC™ Baculovirus expression system (Gibco, BRL). The MICA expression vector was constructed by PCR using a previously described MICA cDNA clone as template (Bahram *et al.*, 1994). The coding region for residues 1–299 consisting of signal peptide and the extracellular domains $\alpha1$, $\alpha2$, $\alpha3$ was amplified using the primers,

5′-TCTGGATCCATGGGGCTGGGCCCGGTC-3′

and

5′-CATGAATTCCTACTAATGGTGGTGATGA-TGGTGTTTCCCAGAGGGCACAGGGTGAG-3′.

The 3′ primer contains the coding sequence for six C-terminal histidine residues. The resulting fragment was cut with the restriction enzymes *Bam*HI and *Eco*RI and cloned into pFASTBAC1 (Gibco BRL). Error-free cDNA clones were identified by sequencing and used to produce recombinant Baculovirus in Sf9 cells (ATCC CRL-1711) following the manufacturer's protocol.

Sf9 and High-Five™ cells were maintained in serum-free SF 900-II medium (Gibco, BRL). For protein production

Table 1. *Data statistics*

	MICA native data set	
Cryopreservative	Glycerol	Sucrose
Completeness (∞–3.15 Å) (%)	94.9	93.9
Completeness (3.20–3.15 Å) (%)	72.0	48.1
R_{symm} (∞–3.15 Å) (%)†	6.8	8.3
R_{symm} (3.20–3.15 Å) (%)†	21.2	33.9
Redundancy	7.8	10.4

† R_{symm} is defined as $\sum(|I - \bar{I}|)/\sum \bar{I}$.

High-Five™ cells were seeded at a cell density of 3×10^6 cells ml^{-1} and infected with recombinant virus. Medium was typically harvested at day 4 or 5 after infection and MICA was purified from culture supernatant by affinity chromatography. Affinity purification was performed on Ni^{2+}-charged Chelating Sepharose Fast Flow resin (Pharmacia/LKB) following the manufacturer's protocol. Eluted protein was further purified by size-exclusion chromatography (Superdex 75 16/60 column, Pharmacia/LKB) in 50 m*M* PIPES, 150 m*M* NaCl, 1 m*M* EDTA, 0.02% NaN_3, pH 7.0. Isolated MICA protein was concentrated to 20 mg ml^{-1} using Centricon ultrafilters (Amicon).

2.2. *Crystallization*

All crystallization trials were conducted by vapor diffusion in a hanging-drop geometry (Crystal Systems Q Plates™; Hampton Research) at a constant temperature of 291 K. Drops consist of equal volumes of concentrated protein solution and well solution (well volume = 1.0 ml). The well solution for optimal growth of MICA crystals contains 50–55% saturated ammonium sulfate buffered with 50–100 m*M* sodium citrate (pH = 5.5). Crystals grow over the course of a week (see Fig. 1). For cryocrystallography, either glycerol or sucrose (Fisher Scientific) can be added to the growth conditions in concentrations of up to either 15%(*v*/*v*) glycerol or 15%(*w*/*w*) sucrose without significantly impacting visible crystal quality.

2.3. *Crystallography*

For X-ray analysis, crystals were mounted in rayon loops (Woolworth) on magnetic pins [a variation of the methods of Teng (1990) and Rodgers (1994)] and flash-cooled to 103 K (Molecular Structure Corporation Low-Temperature System). Diffraction is not observed when crystals are mounted conventionally in capillaries and photographed at room temperature. Optimal cryo-preservation of MICA crystals requires the presence of 25%(*v*/*v*) glycerol or 25%(*w*/*w*) sucrose. Crystals grown in intermediate concentrations of these cryo-protectants are transferred gradually to solutions containing the final concentration by moving crystals between drops containing mother liquor and increasing concentrations of cryo-protectant in steps of 2% every 15 min. Diffraction data were measured with a Rigaku R-AXIS IIc image-plate detector mounted on a Rigaku RU-200 generator with a 300 µm focusing cup, a graphite monochromator and a copper anode operating at 100 mA and 50 kV. Initial space-group assignments, lattice constant determinations and data reduction were conducted with *DENZO* (Otwinowski, 1991). The presence of the 4_1 screw element was diagnosed by inspection of the reduced data (in *F*432) for systematic absences beyond those resulting from the centering operations. Data were collected as 0.8° oscillations, 2 h exposures, at crystal-to-detector distances of between 165 and 190 mm.

3. Results and discussion

Crystals of human MICA were grown from ammonium sulfate at low pH, and have the appearance of truncated cubes which can grow as large as 1 mm on an edge. These crystals do not diffract at room temperature in conventional mounts. MICA crystals can be flash-cooled in a nitrogen gas stream to 103 K in cryo-buffers containing either 25%(*w*/*w*) sucrose or 25%(*v*/*v*) glycerol. Cryo-preserved MICA crystals diffract to a d_{min} of approximately 3.0 Å. The space group and lattice constants were determined by processing 0.8° oscillation diffraction patterns taken from cryo-preserved crystals and by the presence of the *h*00: $h = 4n$ selection rule. The MICA crystals belong to the cubic space group $F4_132$ (consistent with their morphology) with lattice constants $a = b = c = 260.7$ Å. Based on calculations of solvent content, the crystals have either one (solvent content $\simeq$ 77%) or two (solvent content $\simeq$ 37%) molecules in the asymmetric unit. Native diffraction data sets have been collected from cryo-preserved MICA crystals with either glycerol or sucrose as the cryo-protectant. Both data sets are essentially complete to a resolution of 3.15 Å and are high quality (see Table 1). Crystals used for these data sets measured approximately 350 µm along one edge and displayed mosaicities of approximately 0.4°. Larger crystals do not diffract to higher resolutions and generally display higher mosaicities (up to 1°) prohibiting data collection with a unit cell of this size on this equipment. We are currently conducting both molecular replacement and

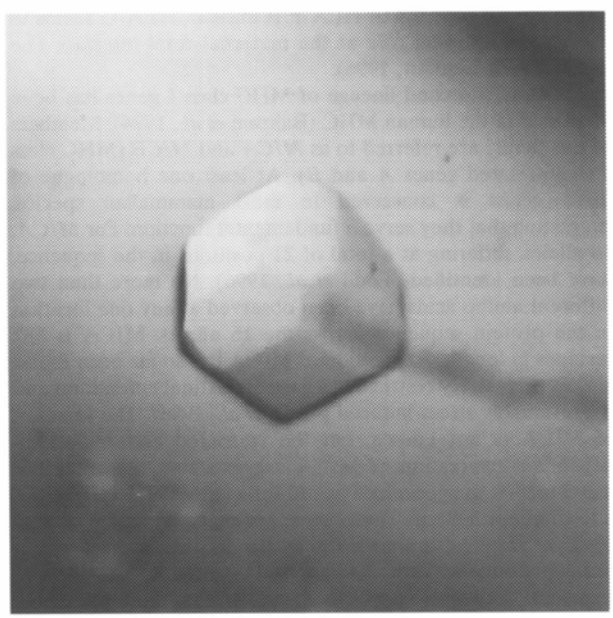

Fig. 1. A crystal of human MICA grown from ammonium sulfate at low pH. The dimensions are approximately 450 × 450 × 450 µm.

multiple isomorphous replacement trials in order to obtain an initial phase set. The former ultilizes the many class I and class II crystal structures currently available (treating 'platform' domains and immunoglobulin domains as separate search objects to account for possible domain rearrangements) and the latter focuses on mercurial compounds to take advantage of the single free sulfhydryl present in the MICA sequence.

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