Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of recombinant full-length human m-calpain

Hajime Masumoto,[a] Kazuhiro Nakagawa,[a] Shota Irie,[a] Hiroyuki Sorimachi,[a] Koichi Suzuki,[a] Gleb P. Bourenkov,[b] Hans Bartunik,[b] Carlos Fernandez-Catalan,[c] Wolfram Bode[c]* and Stefan Strobl[c]

[a]Laboratory of Molecular Structure and Function, Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan, [b]AG Proteindynamik MPG-ASMB, c/o DESY, D-22603 Hamburg, Germany, and [c]Abteilung für Strukturforschung, Max-Planck-Institut für Biochemie, Am Klopferspitz 18a, D-82152 Martinsried, Germany

Correspondence e-mail: bode@biochem.mpg.de

Received 18 August 1999
Accepted 25 October 1999

m-Calpain constitutes the prototype of the superfamily of neutral calcium-activated cysteine proteinases. It is a heterodimer consisting of an 80 and a 30 kDa subunit. Recombinant full-length human m-calpain has been crystallized using macro-seeding techniques and vapour-diffusion methods. Two different monoclinic crystal forms (space group $P2_1$) were obtained from a solution containing polyethylene glycol (M_W = 10 000) as a pecipitating agent. Complete data sets have been collected to 2.3 and 3.0 Å resolution using cryo-cooling conditions and synchrotron radiation. The unit-cell parameters are a = 64.86, b = 133.97, c = 78.00 Å, β = 102.43° and a = 51.80, b = 171.36, c = 64.66 Å, β = 94.78°, respectively. The V_m values indicate that there is one heterodimer in each asymmetric unit.

1. Introduction

Calpain is a Ca^{2+}-activated neutral cysteine proteinase expressed in the cytosol of higher animal cells. Calpains are classified into two groups: the first group consists of ubiquitously expressed calpains, while members of the second group are expressed in specific tissues only (Ono *et al.*, 1998; Sorimachi *et al.*, 1997). Ubiquitously expressed species, such as μ-calpain and m-calpain, are found in animal tissues as heterodimers consisting of a distinct large catalytic subunit (80 kDa) and a small regulatory subunit (30 kDa) common to these calpains. The 80 kDa subunit contains a domain which bears some sequence similarity to the bilobal papain domain (Ohno *et al.*, 1984). Each subunit exhibits a C-terminal calmodulin-like Ca^{2+}-binding domain. In 1997, the crystal structures of the 21 kDa Ca^{2+}-binding domain of the 30 kDa subunit of porcine and rat m-calpain were determined in the absence as well as in the presence of Ca^{2+} (Blanchard *et al.*, 1997; Lin *et al.*, 1997). These crystal structures showed that Ca^{2+} induces conformational changes within the calmodulin-like domains and revealed that there is a fifth EF-hand motif N-terminal in addition to the four EF-hand motifs predicted from amino-acid sequence homologies (Emori *et al.*, 1986). Based on these crystal structures, it was suggested that the two subunits interact *via* their C-terminal EF-hand motifs. How binding of calcium to these calmodulins triggers the active site of the catalytic domain remains completely unclear, however. Determination of the complete quaternary structure with and without Ca^{2+} bound is essential in order to understand the activation mechanism of calpain by Ca^{2+} and its structure–function relationships.

In order to prepare samples for crystallization, we established an expression system for m-calpain by co-expressing the 80 and 30 kDa subunits in a baculovirus system (Masumoto *et al.*, 1998). Here, we report the crystallization and a preliminary X-ray analysis of recombinant full-length human m-calpain crystals in the absence of Ca^{2+}. Although the physiological function of calpain has not yet been completely elucidated, the determination of the three-dimensional structure will provide deeper insight into the function of this still enigmatic proteinase.

2. Materials and methods

2.1. Purification and crystallization

Full-length human m-calpain was over-expressed in a baculovirus expression system and purified as described before (Masumoto *et al.*, 1998). The protein concentration was determined by absorption spectroscopy using a molar extinction coefficient at 280 nm of $A(1\%)$ = 17.2 mg ml^{-1}. Prior to crystallization, the protein was concentrated to approximately 14 mg ml^{-1} and the buffer was changed to 10 m*M* Tris–HCl pH 7.5, 50 m*M* NaCl, 1 m*M* EDTA, 1 m*M* DTE. Initial screening of crystallization conditions resulted in small crystals of <100 µm in the longest dimension with 20% PEG 10 000, 0.1 *M* HEPES/NaOH pH 7.5. However, only in approximately 5–10% of identical crystallization setups did crystals appear after 2–3 d. Variation of the precipitant condition (pH, PEG molecular weight and concentration) and addition of 2-propanol and guanidinium chloride improved crystal size and growth. Crystals large enough for X-ray diffraction experiments were successfully grown in about 20% of the crystallization trials

Table 1
Summary of recombinant full-length human m-calpain crystals.

Parameter	Crystal form I	Crystal form II
Space group	$P2_1$	$P2_1$
Crystal morphology	Plates	Rods/cuboids
Unit-cell parameters (Å, °)	a = 64.78	a = 51.88
	b = 133.25	b = 169.84
	c = 77.53	c = 64.44
	β = 102.07	β = 95.12
V_m (Å^3 Da^{-1})	3.14	2.61
Heterodimers per asymmetric unit	1	1
Estimated solvent content (%)	61	53
Diffraction limit (Å)	3.0	2.3
Rotation for each exposure	0.5	0.4
Time for each image (s)	50	50
Total rotation for a data set (°)	180	132
Number of measured reflections	223726	400680
Number of unique reflections	25010	47236
$R_{\rm merge}$	0.056	0.045
Completeness (%)	96.9	94.8
Last resolution shell (%)	95.2	92.2

by macro-seeding using the hanging-drop and sitting-drop vapour-diffusion methods. 6.6 µl droplets consisting of 4 µl of protein solution, 2 µl of precipitant solution (100 m*M* HEPES/NaOH pH 7.7, 15% PEG 10 000, 2.2% 2-propanol) and 0.6 µl of 1 *M* guanidinium chloride were equilibrated against 400 µl of the precipitant solution at 293 K.

2.2. X-ray diffraction analysis

None of the crystals were stable to X-ray exposure at room temperature for very long. Therefore, we performed a search for a suitable cryo-protectant buffer. Crystals were harvested from the droplets with cryo-loops and transferred into 20 µl of the reservoir buffer. 10 µl of 88% glycerol were slowly added in order to adapt the crystals to cryo-buffer conditions. The equilibrated crystals were mounted in loops and flash-cooled in the gas stream of a liquid-nitrogen cryostat (Oxford Cryosystems Cryostream). X-ray diffraction data were collected at 100 K on a MAR CCD detector at the BW6 wiggler beamline of DORIS (Deutsches Elektonen Synchrotron, Hamburg, Germany) using monochromatic X-ray radiation. The data were indexed with *DENZO* (Otwinowski & Minor, 1993), scaled and reduced with *SCALEPACK* (Otwinowski & Minor, 1993) and truncated with programs from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).

3. Results

3.1. Crystals and X-ray diffraction

Two crystal morphologies were observed under identical conditions, occasionally even within the same droplet. Both crystal forms belong to the monoclinic space group $P2_1$. A summary of the two crystal forms is provided in Table 1.

3.2. Crystal form I

Crystal form I is a rhombic platelet with unit-cell parameters a = 64.78, b = 133.25, c = 77.53 Å, β = 102.07°. The crystals grow to maximum dimensions of 1.0 × 1.0 × 0.1 mm (Fig. 1) and show Bragg spacing to 2.75 Å resolution. From native crystals, a 3.0 Å data set was collected over 360 frames (0.5° oscillation range, 50 s exposure time). According to the V_m value of 3.14 Å^3 Da^{-1} (Matthews, 1968), there should be one heterodimer in the asymmetric unit, corresponding to a solvent content of 61%(*v*/*v*). A total of 223 726 reflections were recorded and merged to obtain 25 010 unique reflections ($R_{\rm merge}$ = 5.6%), corresponding to 96.9% of the theoretically possible reflections.

3.3. Crystal form II

Crystal form II has a platelet-like to prism-like morphology with unit-cell parameters a = 51.88, b = 169.84, c = 64.44 Å, β = 95.12°. The crystals grow to maximum dimensions of 1.0 × 0.2 × 0.1 mm (Fig. 2) and show Bragg spacings to 2.1 Å resolution. From crystals soaked in 5 m*M* chlorotriethylphosphine gold, a 2.3 Å data set was collected over 330 frames (0.4° oscillation range, 50 s exposure time). Based on the V_m value of 2.61 Å^3 Da^{-1} (Matthews, 1968), these crystals contain one heterodimer per asymmetric unit, corresponding to a solvent content of 53%(*v*/*v*). A total of 400 680 reflections were recorded and merged to obtain 47 236 unique reflections ($R_{\rm merge}$ = 4.5%), corresponding to 94.8% of the theoretically possible reflections.

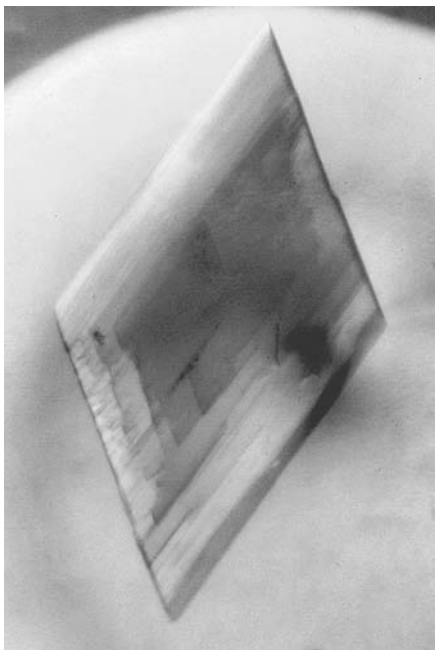

Figure 1
Monoclinic crystal of human m-calpain (crystal form I) with approximate dimensions 1.0 × 1.0 × 0.1 mm.

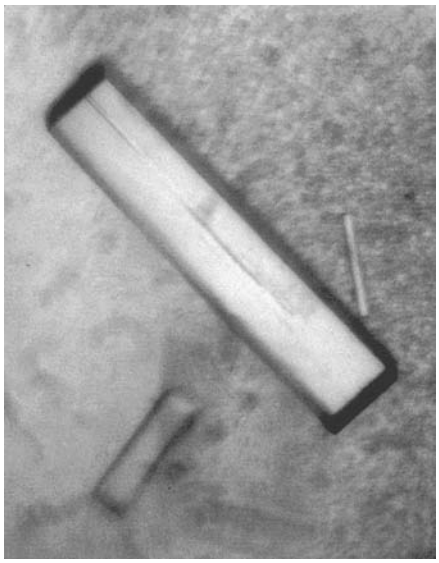

Figure 2
Monoclinic crystal of human m-calpain (crystal form II) with approximate dimensions 1.0 × 0.2 × 0.1 mm.

4. Discussion

Successful crystallization and initial data collection are the first steps in determining the three-dimensional structure of human m-calpain. m-Calpain constitutes the prototype of the superfamily of neutral Ca^{2+}-activated cysteine proteinases. At the primary structure level, it shows a distinct domain organization (Ohno *et al.*, 1984; Sorimachi *et al.*, 1997). The 80 kDa subunit consists of a small prodomain, a cysteine proteinase domain expected to be papain-like, a domain of unknown function which might be involved in substrate recognition, and the Ca^{2+}-binding domain, which seems to be involved in heterodimer formation with the corresponding domain of the 30 kDa subunit. The small subunit, in addition to its Ca^{2+}-binding domain, contains a hydrophobic glycine-rich N-terminal part which is considered to interact with membranes or membrane-associated proteins *via* hydrophobic interactions (Imajoh *et al.*, 1986).

In contrast to other heterologously expressed m-calpain constructs (Elce *et al.*, 1995), the full-length human m-calpain we have used for crystallization contains the glycine-rich domain of the 30 kDa subunit. We produced two crystal forms with different packing geometries, which might help to analyze the intrinsic conformation of the domain arrangement and to distinguish functional important conformations from packing artefacts. Thus, the two crystal forms will be particularly useful in helping to explain the activation mechanism, membrane interaction and substrate recognition.

We are searching for heavy-atom derivatives and have already found promising candidates. Together with the established cryo-cooling conditions and access to synchrotron radiation, determination of the high-resolution structure of m-calpain will be feasible in the near future.

References

Blanchard, H., Grochulski, P., Li, Y., Arthur, J. S. C., Davies, P. L., Elce, J. S. & Cygler, M. (1997). *Nature Struct. Biol.* **4**, 532–538.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Elce, J. S., Hegadorn, C., Gauthier, S., Vince, J. W. & Davies, P. L. (1995). *Protein Eng.* **8**, 843–848.

Emori, Y., Kawasaki, H., Imajoh, S., Imahori, K. & Suzuki, K. (1986). *J. Biol. Chem.* **261**, 9472–29476.

Imajoh, S., Kawasaki, H. & Suzuki, K. (1986). *J. Biochem.* **99**, 1281–1284.

Lin, G., Chattopadhyay, D., Maki, M., Wang, K. K. W., Carson, M., Jin, L., Yuen, P., Takano, E., Hatanaka, M., Delucas, L. J. & Narayana, S. V. L. (1997). *Nature Struct. Biol.* **4**, 539–547.

Masumoto, H., Yoshizawa, T., Sorimachi, H., Nishino, T., Ishiura, S. & Suzuki, K. (1998). *J. Biochem.* **124**, 957–961.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Ohno, S., Emori, Y., Imajoh, S., Kawasaki, H., Kisaragi, M. & Suzuki, K. (1984). *Nature (London)*, **312**, 566–570.

Ono, Y., Sorimachi, H. & Suzuki, K. (1998). *Biochem. Biophys. Res. Commun.* **245**, 289–294.

Otwinowski, Z. & Minor, W. (1993). *DENZO. A Film Processing Program for Macromolecular Crystallography*. Yale University, New Haven, Connecticut, USA.

Sorimachi, H., Ishiura, S. & Suzuki, K. (1997). *Biochem. J.* **328**, 721–732.