Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray crystallographic analysis of human peptidylarginine deiminase V

Kyouhei Arita,[a] Hiroshi Hashimoto,[a] Toshiyuki Shimizu,[a] Michiyuki Yamada[b] and Mamoru Sato[a]*

[a]Graduate School of Integrated Science, Yokohama City University, 1-7-29 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan, and [b]Graduate School of Integrated Science, Yokohama City University, 22-2 Seto, Kanazawa-ku, Yokohama 236-0027, Japan

Correspondence e-mail: msato@tsurumi.yokohama-cu.ac.jp

Received 6 October 2003
Accepted 9 October 2003

Human peptidylarginine deiminase V (PAD V) is a post-translational enzyme that catalyzes the conversion of arginine residues in protein into citrulline residues in the presence of calcium ion. Crystals of PAD V have been grown at 293 K using polyethylene glycol monomethylether as a precipitant. Crystals diffracted beyond 2.7 Å resolution at 100 K at the SPring-8 synchrotron-radiation source. The crystal belongs to space group $C2$, with unit-cell parameters $a = 144.6$, $b = 60.4$, $c = 113.4$ Å, $\beta = 123.6^\circ$. The asymmetric unit contains one molecule, with a V_M of 2.56 Å^3 Da^{-1} and a solvent content of 56.1%. A full set of X-ray diffraction data was collected to 2.8 Å resolution with a completeness of 97.5%. Heavy-atom derivatives have been successfully prepared and structure analysis is in progress.

1. Introduction

Peptidylarginine deiminase (PAD; EC 3.5.3.15) catalyzes the conversion of arginine residues in protein into citrulline residues in the presence of calcium ions. There are four types (types I, II, III and V) of PAD in human tissues (Guerrin *et al.*, 2003; Ishigami *et al.*, 2002; Kanno *et al.*, 2000; Nakashima *et al.*, 1999). These enzymes show different substrate specificities and tissue distributions. Biochemical and immunocytochemical studies have suggested that PAD I is involved in the terminal differentiation of epidermis (Senshu *et al.*, 1999), PAD II in the myelination and demyelination of central nerve axons (Pritzker *et al.*, 2000) and PAD III in the keratinization of hair follicles (Rogers *et al.*, 1999).

PAD V was first found in human myeloid leukemia HL-60 cells induced to differentiate into granulocytes by all-*trans* retinoic acid. Subsequently, this enzyme was also found in peripheral blood granulocytes. PAD V in HL-60 cells (Nakashima *et al.*, 1999) and pheripheral blood granulocytes (Asaga *et al.*, 2001) can be activated to deiminate the nuclear protein of nucleophosmin/B23 and core histones of H2A, H3 and H4 by stimulation with calcium ionophore (Hagiwara *et al.*, 2002). PAD V is unique among the PAD isoforms in that it is localized in the nucleus with a nuclear localization signal motif (^{56}PPAKKKST^{63}; Nakashima *et al.*, 2003). A recent case-control linkage disequilibrium study using single-nucleotide polymorphisms (SNPs) showed that individuals with rheumatoid arthritis frequently have autoantibodies to citrullinated peptides and the gene that codes PAD V is a susceptibility locus for rheumatoid arthritis, suggesting the involvement of the PAD V in rheumatoid arthritis (Suzuki *et al.*, 2003).

To elucidate the precise molecular mechanism of protein deimination by PAD V and to design new drugs that inhibit protein deimination for treatment of rheumatoid arthritis, we initiated the X-ray crystal structure analysis of PAD V. Here, we report the crystallization and preliminary X-ray crystallographic analysis of human PAD V as the first step of the structure analysis.

2. Experimental

2.1. Expression and purification

Recombinant human PAD V was expressed in *Escherichia coli* BL21 (DE3) cells transformed with plasmid pGEX6P-1 (Amersham Biosciences) as a fusion protein with glutathione *S*-transferase (GST). The cells were disrupted by sonication at 277 K. The supernatant was applied to a DEAE Sepharose CL-6B anion-exchange column (Amersham Biosciences) equilibrated with 20 m*M* Tris–HCl pH 8.0 buffer containing 1 m*M* EDTA, 1 m*M* dithiothreitol (DTT) and 10% glycerol. The adsorbed fraction was eluted with equilibrium buffer containing 200 m*M* NaCl. The eluted fractions were applied to a glutathione Sepharose 4B GST affinity column (Amersham Biosciences) equilibrated with 20 m*M* Tris–HCl pH 8.0 buffer containing 200 m*M* NaCl, 1 m*M* EDTA, 1 m*M* dithiothreitol (DTT) and 10% glycerol. The adsorbed fraction was eluted with equilibrium buffer containing 15 m*M* reduced glutathione. The fusion protein was cleaved with PreScission Protease (Amersham Biosciences) at 200 units per 2 l of culture for 24 h at 277 K. The cleaved proteins were applied to a HiTrap Q anion-exchange column (Amersham Biosciences) equipped with an ÄKTA FPLC system equilibrated with

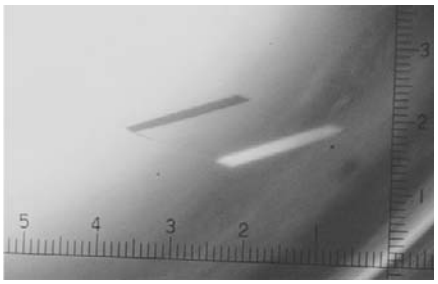

Figure 1
A crystal of PAD V. The approximate dimensions of the crystal are 0.4 × 0.2 × 0.03 mm.

20 m*M* Tris–HCl pH 8.5 buffer at 277 K. The adsorbed fraction was eluted with a linear gradient of 100–400 m*M* NaCl with 10 m*M* Tris–HCl pH 8.5 buffer at 277 K. The homogeneity of the purified protein was confirmed by SDS–PAGE.

2.2. Crystallization

Purified protein in 10 m*M* Tris–HCl pH 8.5 buffer containing 500 m*M* NaCl, 1 m*M* DTT and 1 m*M* EDTA was concentrated to about 4 mg ml^{-1} in a Centricon-50 concentrator (Amicon) at 277 K. Crystallization was performed by the hanging-drop vapour-diffusion method using a 24-well tissue-culture plate (Sumitomo Bakelite Co.). Preliminary screening of crystallization conditions was performed using Hampton Research Crystal Screen kits and Emerald Biostructure Screen kits at 277 and 293 K. Small crystals were obtained using Wizard Screen solution No. 15 containing 0.1 *M* imidazole pH 8.0, 0.2 *M* lithium sulfate and 10%(*w*/*v*) polyethylene glycol monomethylether (PEGMME) 5000 at 293 K. Refinement of the crystallization conditions to 0.1 *M* imidazole pH 8.0, 0.2 *M* lithium sulfate and 8%(*w*/*w*) PEGMME 2000 greatly improved the quality and size of the crystals.

2.3. Data collection and processing

Crystals were soaked in crystallization buffer containing 20% ethylene glycol as a cryoprotectant prior to the X-ray experiment. X-ray diffraction data from the native crystal were collected at 100 K on the ADSC Quantum 4R CCD system installed at BL38B1, SPring-8, Japan. The diffraction data were processed using *CrystalClear* (Pflugrath, 1999).

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell (2.9–2.8 Å).

X-ray source	SPring-8 BL38B1
Wavelength (Å)	1.000
Space group	*C*2
Unit-cell parameters	
a (Å)	144.6
b (Å)	60.4
c (Å)	113.6
β (°)	123.6
Resolution range (Å)	33.42–2.8
$R_{\rm merge}$† (%)	4.6 (26.1)
Average $I/\sigma(I)$	12.7 (3.1)
No. observations	93345
No. unique reflection	38401
Data completeness (%)	97.6 (98.3)
Redundancy	2.39 (2.40)
Crystal mosaicity (°)	0.79

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all measured reflection and $\sum_i$ is the sum over i measurements of a reflection.

3. Results

Crystals suitable for high-resolution X-ray crystallographic analysis were obtained at 293 K in a few days when drops containing equal volumes (3–7 µl) of protein (4 mg ml^{-1}) and reservoir solution [0.1 *M* imidazole pH 8.0, 0.2 *M* lithium sulfate and 8%(*w*/*w*) PEGMME 2000] were equilibrated against 0.2 ml of reservoir solution (Fig. 1).

The crystal belongs to space group *C*2, with unit-cell parameters a = 144.6, b = 60.4, c = 113.4 Å, β = 123.6°. The presence of one PAD V molecule with a molecular weight of 74.1 kDa in the asymmetric unit gives a $V_{\rm M}$ value of 2.56 Å^3 Da^{-1} and a solvent content of 56.1%, which lie within the ranges usually found for protein crystals (Matthews, 1968). Crystals diffracted beyond 2.7 Å resolution at 100 K. A full set of X-ray diffraction data was collected to 2.8 Å resolution with a completeness of 97.5%. Table 1 summarizes the data-collection statistics of the native crystal.

Attempts to prepare heavy-atom derivatives of the native crystal for phase determination have been performed and some heavy-atom derivatives have been successfully prepared using the soaking method. Structure determination by the multiple isomorphous replacement anomalous scattering (MIRAS) method is in progress.

We thank the staff at beamline BL38b1, SPring-8, Japan for data-collection facilities and support. This work was supported by Grants-in-Aid for Young Scientists (B) from Japan Society of the Promotion of Science (JSPS) to HH (14780515), a Grant-in-Aid for Scientific Research (C) from JSPS to TS (15570101) and the Protein 3000 Project from The Ministry of Education, Culture, Sports, Science and Technology (MEXT).

References

Asaga, H., Nakashima, K., Senshu, T., Ishigami, A. & Yamada, M. (2001). *J. Leukoc. Biol.* **70**, 46–51.
Guerrin, M., Ishigami, A., Mechin, M. C., Nachat, R., Valmary, S., Sebbag, M., Simon, M., Senshu, T. & Serre, G. (2003). *Biochem. J.* **370**, 167–174.
Hagiwara, T., Nakashima, K., Hirano, H., Senshu, T. & Yamada, M. (2002). *Biochem. Biophys. Res. Commun.* **290**, 979–983.
Ishigami, A., Ohsawa, T., Asaga, H., Akiyama, K., Kuramoto, M. & Maruyama, N. (2002). *Arch. Biochem. Biophys.* **407**, 25–31.
Kanno, T., Kawada, A., Yamanouchi, J., Yosida-Noro, C., Yoshiki, A., Shiraiwa, M., Kusakabe, M., Manabe, M., Tezuka, T. & Takahara, H. (2000). *J. Invest. Dermatol.* **115**, 813–823.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Nakashima, K., Hagiwara, T., Ishigami, A., Nagata, S., Asaga, H., Kuramoto, M., Senshu, T. & Yamada, M. (1999). *J. Biol. Chem.* **274**, 27786–27792.
Nakashima, K., Hagiwara, T. & Yamada, M. (2003). *J. Biol. Chem.* **277**, 49562–49568.
Pflugrath, J. W. (1999). *Acta Cryst.* D**55**, 1718–1725.
Pritzker, L. B., Joshi, S., Harauz, G. & Moscarello, M. A. (2000). *Biochemistry*, **39**, 5382–5388.
Rogers, G., Winter, B., McLaughlan, C., Powell, B. & Nesci, A. (1999). *Exp. Dermatol.* **8**, 362–363.
Senshu, T., Akiyama, K., Ishigami, A. & Nomura, K. (1999). *J. Dermatol Sci.* **21**, 113–126.
Suzuki, A. *et al.* (2003). *Nature Genet.* **34**, 395–402.