Masaki Unno,^{a,b,c,*} Saya Kinjo,^d
Kenji Kizawa^e and Hidenari
Takahara^d

^aFrontier Research Center for Applied Atomic
Sciences, Ibaraki University, 162-1 Shirakata,
Tokai, Naka, Ibaraki 319-1106, Japan,

^bGraduate School of Science and Engineering,
Ibaraki University, 4-12-1 Nakanarusawa,
Hitachi, Ibaraki 316-8511, Japan, ^cInstitute for
Protein Research, Osaka University, 3-2
Yamadaoka, Suita, Osaka 565-0871, Japan,

^dDepartment of Applied Biological Resource
Sciences, Ibaraki University, 3-21-1 Chuo, Ami,
Inashiki, Ibaraki 300-0393, Japan, and

^eInnovative Beauty Science Laboratory, Kanebo
Cosmetics Inc., 3-3-28 Kotobuki, Odawara,
Kanagawa 250-0002, Japan

Correspondence e-mail:
unno19@mx.ibaraki.ac.jp

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

Peptidylarginine deiminase (PAD) catalyzes the post-translational conversion of peptidylarginine to peptidylcitrulline in the presence of calcium ions. Among the five known human PAD isozymes (PAD1–4 and PAD6), PAD1 exhibits the broadest substrate specificity. Crystals of PAD1 obtained using polyethylene glycol 3350 as a precipitant diffracted to 3.70 Å resolution using synchrotron radiation. Two PAD1 molecules were contained in the asymmetric unit and the crystals belonged to space group $P6_1$, with unit-cell parameters $a = b = 90.3$, $c = 372.3$ Å. The solvent content was 58.2%.

1. Introduction

Peptidylarginine deiminase (PAD; EC 3.5.3.15) is a calcium-dependent enzyme that catalyzes the conversion of arginine residues to citrulline residues in proteins (Vossenaar *et al.*, 2003). This irreversible post-translational modification alters the intermolecular and intramolecular interactions of target proteins (Ying *et al.*, 2009). In humans, the five known PAD genes (*PADI1*–4 and *PADI6*) are located within a single cluster on chromosome 1p36.1 and are differentially expressed during tissue development and cellular differentiation: human PAD1–3 proteins are expressed during epithelial differentiation (Kanno *et al.*, 2000; Nachat *et al.*, 2005), while PAD4 is expressed in haematopoietic cells and PAD6 is expressed in the ovary and testis (Vossenaar *et al.*, 2003).

Previous studies have determined the target sites of PAD enzymes in several proteins and have revealed the consequences of deimination. Co-localization studies of PADs with substrate proteins have suggested that PAD1–3 deiminate filaggrin and keratins in the skin epidermis (Chavanas *et al.*, 2006; Méchin *et al.*, 2007), as well as S100A3 and trichohyalin in hair follicles (Kizawa *et al.*, 2008; Tarcsa *et al.*, 1998). The five human PAD enzymes share approximately 50–55% sequence identity at the amino-acid level (Liu *et al.*, 2011; Chavanas *et al.*, 2004). Although the catalytic residues at the C-terminal regions of all known PAD isozymes are more conservative, their substrate specificities differ markedly. For example, PAD1 and PAD2 convert the four arginine residues in S100A3 (Arg3, Arg22, Arg51 and Arg77) to citrulline residues, whereas PAD3 deiminates only one arginine residue (Arg51), which is involved in homo-tetramerization of S100A3 (Kizawa *et al.*, 2008, 2013).

X-ray crystallographic analyses of human PAD4 (Arita *et al.*, 2004, 2006) and PAD3 (Unno *et al.*, 2012) showed that these isozymes form a dimer with a similar overall structure; however, no structural information is currently available for the other isozymes. To elucidate the catalytic mechanism that confers broad substrate specificity, it is necessary to determine the X-ray structures of PAD1 and PAD2. Here, we report the generation of crystals of the full-length human PAD1 protein comprising 663 amino acids [accession No. Q9ULC6 (*PADI1_HUMAN*) in the UniProtKB database; Guerrin *et al.*, 2003] and describe the results of a preliminary X-ray crystallographic analysis.

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Table 1

Data-collection statistics.

Values in parentheses are for the outermost resolution shell.

X-ray source	BL-5A, Photon Factory
Wavelength (Å)	0.9
Space group	$P6_1$
Unit-cell parameters (Å)	$a = b = 90.3$, $c = 372.3$
Resolution range (Å)	50.0–3.70 (3.76–3.70)
$R_{\text{merge}}^{\dagger}$ (%)	10.9 (48.3)
Average $I/\sigma(I)$	27.6 (5.55)
No. of observations	211539 (10962)
No. of unique reflections	18298 (945)
Completeness (%)	99.8 (100)
Multiplicity	11.6 (11.6)
Crystal mosaicity (°)	0.71

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the i th observation of reflection hkl , $\langle I(hkl) \rangle$ is the average intensity of reflection hkl , $\sum_{hkl}$ is the sum over all the measured reflections and $\sum_i$ is the sum over i measurements of a reflection.

2. Experimental

2.1. Protein expression and purification

The recombinant vector containing 6×His-tagged human PAD1 (pET-16b-PAD1) was a kind gift from Professor P. R. Thompson (University of South Carolina) (Knuckley *et al.*, 2010). The His-tagged PAD1 protein was expressed in *Escherichia coli* BL21(DE3)pLysS cells and purified using a His-tag affinity column, as reported previously for PAD3 (Unno *et al.*, 2012). Briefly, cells cultured in 500 ml Luria Bertani medium were disrupted by sonication at 277 K in buffer A [20 mM Tris–HCl buffer pH 7.6, 400 mM NaCl, 10% (v/v) glycerol, 10 mM β -mercaptoethanol] supplemented with 1 mM phenylmethanesulfonyl fluoride. After centrifugation at 40 000g for 20 min, the supernatant was loaded onto a 5 ml Ni²⁺-charged HiTrap Chelating HP column (GE Healthcare) that had been equilibrated with buffer A supplemented with 20 mM imidazole. The column was then washed with ten column volumes of buffer A supplemented with 20 mM imidazole. The adsorbed His-tagged protein was eluted with a linear (20–500 mM) imidazole gradient and then concentrated to a volume of 2.5 ml using an Amicon Ultra-4 centrifugal 10K filter device (Millipore). The condensed sample was applied onto a HiPrep 16/60 Sephacryl S-200 HR gel-filtration column (GE Healthcare) that had been pre-equilibrated with three column volumes of buffer B [20 mM Tris–HCl pH 7.6, 400 mM NaCl,

10% (v/v) glycerol, 1 mM EDTA, 2 mM dithiothreitol] and then calibrated using a Gel Filtration High Molecular Weight Calibration Kit (GE Healthcare). A major peak of approximately 110 kDa was identified in the eluate. The homogeneity of the purified His-tagged PAD1 protein (76 kDa) was confirmed by SDS–PAGE. All liquid chromatography was performed using the ÄKTAprius plus chromatography system (GE Healthcare).

2.2. Crystallization

Purified human PAD1 protein was successfully crystallized without His-tag cleavage using the hanging-drop vapour-diffusion method. Purified protein in buffer B was concentrated to 3.5–8.0 mg ml^{−1} by centrifugation at 277 K. Preliminary screening of crystallization conditions was performed using commercially available screening kits (Hampton Research). The concentrated protein solution (1 μ l) was mixed with an equal volume of crystallization solution and the drops were then equilibrated at 293 K against a 200 μ l reservoir of crystallization solution in a 24-well tissue culture plate (Falcon). Small crystals were obtained using PEG/Ion screen solution No. 7, which consisted of 0.2 M CaCl₂, 20% (w/v) polyethylene glycol 3350. Crystals of improved quality and size were reproducibly obtained from a mixture of 2 μ l protein solution and an equal volume of crystallization solution consisting of 50 mM citric acid buffer pH 6.0, 0.2 M CaCl₂, 20% (w/v) polyethylene glycol 3350 (Fig. 1).

2.3. Data collection and processing

Prior to X-ray diffraction, the crystals were soaked in crystallization solution supplemented with 25% (v/v) glycerol as a cryoprotectant. X-ray diffraction data were collected at 100 K using an ADSC Quantum 315r CCD detector installed on beamline BL-5A at the Photon Factory, Tsukuba, Japan. The oscillation angle, beam-slit size, exposure time, X-ray transmission and wavelength were 1°, 0.2 mm (horizontal) $\times$ 0.2 mm (vertical), 5 s, 100% and 1.00 Å, respectively. A total of 180 images were collected. The diffraction data were processed using the HKL-2000 software package (Otwinowski & Minor, 1997).

3. Results and future work

Full-length His-tagged PAD1 crystallized within 1 week at 293 K and diffracted to 3.70 Å resolution. The crystals belonged to space group $P6_1$, with unit-cell parameters $a = b = 90.3$, $c = 372.3$ Å, and contained two PAD1 molecules in an asymmetric unit. The Matthews coefficient (V_M) was 2.94 Å³ Da^{−1}, which corresponds to a solvent content of 58.2% (Matthews, 1968). Table 1 summarizes the data-collection statistics of the best crystal obtained. We are currently determining the detailed molecular structure of PAD1, and a structural comparison with PAD3 will be reported in the near future.

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Figure 1

A hexagonal bipyramid-shaped crystal of PAD1 obtained using polyethylene glycol 3350 as a precipitant.

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