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Crystallization and preliminary X-ray diffraction analysis of protein L-isoaspartyl *O*-methyltransferase from wheat germ

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Wheat-germ protein L-isoaspartyl *O*-methyltransferase (WPIMT) can initiate the conversion of L-isoaspartyl residues in a protein or peptide, which accumulate during the aging process in wheat-germ seeds, to normal L-aspartyl groups. The recombinant protein of WPIMT was overexpressed in *Escherichia coli* and purified to homogeneity. The protein was crystallized in the presence of *S*-adenosine-L-homocysteine using 2-methyl-2,4-pentanediol. Preliminary X-ray analysis indicated a tetragonal space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 77.3$, $c = 152.9$ Å for cryofrozen crystals at 90 K. The crystals diffracted to 3.3 Å and contain two molecules per asymmetric unit.

1. Introduction

Protein methyltransferases are widely distributed in nature and catalyze the transfer of a methyl group from L-*S*-adenosyl methionine (AdoMet) to the various amino-acid residues of proteins. These common post-translational modifications create a variety of products exhibiting diverse functions (Clarke, 1992). One such function, the recognition and repair of aged and damaged proteins, is characteristic of type II protein carboxyl methyltransferases, also referred to as protein L-isoaspartyl *O*-methyltransferases (PIMTs; E.C. 2.1.1.77; Fu *et al.*, 1991).

As cells and tissues age, many of their proteins undergo spontaneous changes. One significant change is an alteration of aspartyl and asparaginyl residues, where deamidation, racemization and isomerization occur forming intermediate L-aspartylsuccinimidyl residues and eventually L- and D-isoaspartyl groups. These alterations can substantially affect the normal function of cellular proteins and the cumulative effect can be impairment of cellular function (Clarke, 1992). PIMTs specifically recognize and subsequently methylate the α-carboxyl group of the isoaspartyl residue, allowing the succinimidyl intermediate to reform. A significant portion of these will convert back to L-aspartyl residues (Johnson *et al.*, 1987).

PIMTs are found in mammals, bacteria and plants (Dilberto & Axelrod, 1976). Methyltransferase activity in plants is found in many tissues, but the greatest concentration is found in seeds, where susceptibility to the aging process is immense. Villiers (1975) has proposed that when ample moisture and adequate oxygen are present, protein repair may take place in seeds either at germination or even during dormancy. Mudgett & Clarke (1993) have proposed that protein L-isoaspartyl *O*-methyltransferase plays a role in this repair process. In order to better understand the repair pathway, we have initiated the crystal structure determination of this enzyme. Here, we report the crystallization and preliminary X-ray diffraction analysis of WPIMT.

2. Materials and methods

2.1. Expression and purification

E. coli cells harboring a plasmid containing the WPIMT coding sequence under the control of a T7 promoter were grown in Luria–Bertani broth containing ampicillin, chloramphenicol and 0.2% glucose until $OD_{600} = 0.6$. Isopropyl β-D-thiogalactopyranoside was then added to a final concentration of 1 m*M* and the cells were grown for an additional 4 h. The cells were resuspended in buffer *A* (50 m*M* Tris acetate, 0.1 *M* NaCl, 1 m*M* benzamidine hydrochloride, 0.25 m*M* PMSF, 2.5 m*M* β-mercaptoethanol pH 7.5) and lysed by sonication. The lysates were centrifuged at 30 000 rev min^{-1} for 30 min at 277 K. The protein was precipitated by ammonium sulfate fractionation (70% saturation at 277 K) and the pellet was resuspended in buffer *B* (15 m*M* Tris acetate, 1 m*M* EDTA, 10 m*M* β-mercaptoethanol pH 7.0) and dialyzed for 16 h at 277 K. The dialyzate was applied to a QAE-Sepharose FF column (Pharmacia) equilibrated with buffer *B*. The protein was eluted with a linear gradient of

0–0.6 *M* sodium acetate in buffer *B*. The selected fractions were dialyzed in buffer *C* (25 m*M* Tris–HCl, 10 m*M* β-mercaptoethanol pH 7.8) and were then applied to a Mono Q column (Pharmacia) prepared in buffer *C*. The protein was eluted with a linear gradient of buffer *C* containing 0–0.75 *M* sodium acetate and the fractions of interest were applied to a Sephadex G-75 gel-filtration column (Pharmacia) equilibrated with buffer *C*. WPIMT was >95% pure (as judged by SDS–PAGE) and was dialyzed in MES buffer (15 m*M* MES, 2.5 m*M* magnesium chloride, 0.5 m*M* dithiothreitol pH 6.5).

2.2. Crystallization and data collection

The hanging-drop vapor-diffusion method at 277 K was used for the crystallization experiments. WPIMT was incubated with 5–10 m*M* *S*-adenosine-L-homocysteine (Sigma-Aldrich) for 3 h prior to screen setup. 5 µl of 5.6 mg ml^{-1} WPIMT/*S*-adenosine-L-homocysteine was mixed with an equal volume of precipitating solution [0.1 *M* sodium cacodylate pH 7.0, 20–75 m*M* magnesium acetate (Sigma-Aldrich), 62–68%(*v*/*v*) 2-methyl-2,4-pentanediol (Sigma-Aldrich)] and applied to a siliconized cover slip inverted over a well solution containing 1 ml of the precipitating solution in Linbro tissue-culture trays. Single crystals exhibiting a tetrahedral morphology appear within 7 d and reach maximum dimensions of 0.2 × 0.2 × 0.1 mm within a week.

The crystals were transferred to a cryoprotectant buffer consisting of 10 m*M* sodium cacodylate acid pH 7.0 and 70%(*v*/*v*) 2-methyl-2,4-pentanediol. The crystal was picked up with a fiber loop and frozen in a cold nitrogen-gas stream. Data were collected at 90 K with a Rigaku (Molecular Structure Corporation, Houston, Texas, USA) R-AXIS IV image-plate detector using Cu $K\alpha$ radiation from a Rigaku RU-300 rotating-anode X-ray generator operating at 50 kV and 100 mA with cross-coupled parallel optics. The crystal-to-detector distance was 150 mm. A data set to 3.3 Å was collected with 60 min 0.5° oscillation frames over a 76° oscillation range. The *DENZO* and *SCALEPACK* programs were used for data processing and analysis (Otwinowski & Minor, 1996).

Table 1
X-ray diffraction data.

Values in parentheses refer to the outermost resolution shell.

Crystal system	Tetragonal
Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 77.3$, $c = 152.9$
No. of observed reflections	41541
No. of unique reflections	7460
Resolution range (Å)	3.30 (3.45–3.30)
Completeness (%)	99.8 (99.6)
$R_{\rm merge}$ (%)	12.5 (35.5)

3. Results and discussion

WPIMT crystals were obtained from two different forms of recombinant protein: one was the wild type and the other contained an amino-terminal hexa-histidine tag. Both forms of the protein were purified to homogeneity. The molecular weight of WPIMT is approximately 25 kDa as determined by SDS gel electrophoresis. The pI for the histidine-tagged protein is approximately 5.5; it is approximately 4.5 for the non-histidine-tagged form. Crystals were obtained for both forms of WPIMT, but those grown from the histidine-tagged protein were too thin to yield suitable X-ray diffraction. It is to be noted, however, that twinning of these crystals was alleviated by the addition of Al's Oil (Hampton Research, Laguna Niguel, California, USA) over the reservoir solution. The oil acts to slow the rate of vapor diffusion, allowing the growth of single crystals. Crystals obtained with the non-histidine-tagged WPIMT appeared as small aggregates in the absence of optimal magnesium and *S*-adenosine-L-homocysteine concentrations. When optimal concentrations of both additives were achieved, the crystals exhibited a tetragonal morphology. A complete data set was collected on these crystals to 3.3 Å at 90 K (Table 1). WPIMT crystals are tetragonal and belong to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 77.3$, $c = 152.9$ Å. The value of $V_{\rm M}$ is 2.28 Å^3 Da^{-1} assuming two molecules per asymmetric unit. This corresponds to a solvent content of 46% (Matthews, 1968). Future plans include co-crystallization of WPIMT with its substrate analog, an L-isoaspartyl-containing polypeptide (Research Genetics). A search for heavy-atom derivatives is also in progress.

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