BL17A/2010G616

Crystal structure analysis of mouse SMP30

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1 Introduction

Senescence Marker Protein 30 (SMP30) was originally identified as an ageing marker protein whose expression decreases in an androgen-independent manner in rat liver and kidney cells [1]. Since proteins showing ageing change are typically influenced by hormones, they show different trends of increase or decrease between males and females. However, SMP30 is reduced with ageing in both males and females in the same manner and is not affected by hormones. While SMP30 is involved in biosynthesis of vitamin C in mouse cells, human cells cannot synthesize vitamin C due to mutational inactivation of L-glonolacton-oxygenase, which is the last enzyme of vitamin C biosynthesis. Therefore, human SMP30 (hSMP30) might have a function distinct from vitamin C synthesis. Details of the physiological function of SMP30 in human cells remain elusive.

We aim to elucidate the physiological function of SMP30 and have studied human and mouse SMP30s by comparative biochemistry. Here we report a preliminary crystallographic study of mouse SMP30 (mSMP30).

2 Methods

mSMP30, which shows 88% sequence identity with hSMP30, was crystallized by the sitting-drop vapor diffusion method. 1, 5-Anhydro-D-glucitol was used as a substrate analogue. Crystals of mSMP30 in complex with the substrate analogue were prepared by the soaking method. Diffraction data of mSMP30 crystals were collected at BL-17A of PF at KEK (Table 1). The diffraction data were processed using the program XDS. The crystal structure of mSMP30 was determined by the molecular replacement method with the program MOLREP in CCP4. Since the crystal structure of hSMP30 was already determined [2], it was used as a search model. The crystal structure of mSMP30 was refined using the program Phenix.refine.

3 Results

Mouse SMP30 has the β-propeller structure, which is composed of six β-sheets each of which is formed with four β-strands (Figure 1). At the center of the β-propeller structure, a strong electron density was found. This density was likely to represent a metal ion that was coordinated by three amino residues: Glu18, Asn154, Asp204.

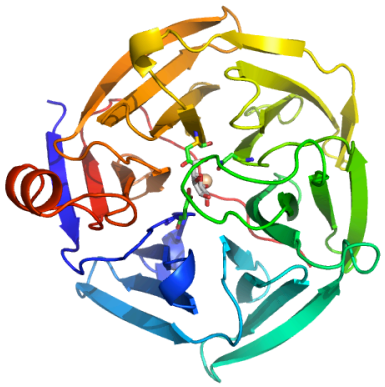

Figure 1 Overall structure of mouse SMP30

Table1 Crystallographic summary

Crystal Form	Substrate free form	Analogue binding form
Beamline	BL17A	BL-17A
Program	XDS	XDS
Wavelength (Å)	0.98	0.98
Oscillation angle (°)	0.5	0.5
Exposure time (s)	4	4
Space group	$P3_121$	$P3_121$
Unit cell parameters		
a	102.68	102.59
c	147.82	149.71
Resolution (Å)	76.2-1.95 (2.06-1.95)	76.4-1.7 (1.79-1.70)
R_{merge} (%)	0.048 (0.424)	0.04 (0.471)
I/σ(I)	22.71 (4.73)	27.18 (4.8)
Completeness (%)	100 (100)	99.9 (100)
No. of unique reflections	127,069 (19,296)	193,833 (27,838)
Redundancy	5.6 (5.7)	5.6 (5.6)
Mosaicity	0.1	0.1

Values in parentheses are for the outermost resolution shell.

4 References

(1) Fujita *et al.*, *Biochim. Biophys. Acta* **1116**, 122-128 (1992).
(2) Chakraborti and Bahnson, *Biochemistry* **49**, 3436-3444 (2010).

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