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Preliminary X-ray diffraction analysis of Senescence Marker Protein-30 (SMP30)

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Introduction

Senescence Marker Protein-30 (SMP30) is an age-associated protein that decreases with aging in an androgen-independent manner. SMP30 is a 34-kd protein expressed mainly in hepatocytes and renal tubular epithelia [1]. Biochemical analyses have suggested that SMP30 participates in Ca^{2+} efflux by activating the calmodulin-dependent Ca^{2+}-pump in HepG2 cells and renal tubular epithelial cells. Moreover, histological and biochemical analyses have indicated that SMP30 has the DFPase activity to decontaminate organo-phosphorus triesters, which act as dangerous chemical warfare agents, e.g. sarin and soman.

To elucidate the molecular mechanism of the SMP30 function, we initiated the X-ray crystal structure analysis of SMP30. Here, we report the crystallization and preliminary X-ray crystallographic analysis of rat SMP30 as the first step of the structure analysis.

Method

SMP30 was purified from rat livers. Crystallization trials were performed by the hanging drop vapor diffusion method at 293K. Droplets were prepared by mixing equal volumes of protein solution and the reservoir solution. Because an initial factorial approach [2] gave no crystals, we used a grid screen techniques using polyethylene glycol as a precipitant. Thin plate-like crystals were grown in three months. To facilitate the crystal growth, we used the micro and macro seeding techniques. The crystals grew to their full size in 1-2 weeks with approximate dimensions of 0.2x0.1x0.03mm^3.

Result

Two crystal forms (Forms I and II) have been obtained from the identical crystallization condition. Forms I and II crystals belong to space groups $P2_1$ and $C2$, respectively.

Data collection of the form I crystal was carried out on the beam line NW12. The diffraction data were processed and scaled using the programs MOSFLM and SCALA in the CCP4 program suite, respectively [3]. The cell parameters of the crystal are a=91.9Å, b=45.2 Å, c=220.1Å and β=101.5°. Assuming four molecules of SMP30 (34kDa) per asymmetric unit, the Matthews coefficient (V_M) was calculated to be 3.3 $Å^3$ Da^{-1}, corresponding to a solvent content of 62.5% (Matthews, 1968).

Data collection of the form II crystal was also carried out on the beam line NW12. The data were processed and scaled using the program HKL2000. The cell parameters of the crystal are a=225.4Å, b=45.4 Å, c=92.6Å and β=98.5°. Assuming two molecules of SMP30 (34kDa) per asymmetric unit, the Matthews coefficient (V_M) was calculated to be 3.4$Å^3$ Da^{-1}, corresponding to a solvent content of 64.0% (Matthews, 1968). More details of the data collection statistics are given in Table 1. An attempt to solve the structure by the method of multiple isomorphous replacement with anomalous scattering (MIRAS) is in progress.

Table 1 Summary of crystallographic data collection and processing

Crystal form	Form I	Form II
Space group	$P2_1$	$C2$
Unit-cell parameters (Å,°)	a=91.9, b=45.2, c=220.1,β=100.5	a=217.7, b=44.9, c=91.7, β=100.1
X-ray source	Photon Factory	Photon Factory
Beamline	NW12	NW12
Wavelength	1.800	1.700
Temperature (K)	100	100
Resolution (Å)	50-2.9	50-2.4
Mosaicity (°)	0.5	0.6
No.of observations	337283	153879
Unique reflections	40372	23085
Completeness	100.0	98.9
Multiplicity	8.4	6.7
Overall I/σ	12.3	11.1
Rsym (%)	7.8	5.5
Rmerge (%)	8.4	6.5

References

[1] T. Fujita et al., *Biochim. Biophys. Acta*, **1116**, 122-128 (1992).

[2] J. Jancarik et al., *J. Appl. Cryst*. **24**, 409-411 (1991).

[3] Collaborative Computational Project, Number 4, *Acta Cryst*. **D50**, 760-763 (1994)

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