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Fbs2: component of SCF complex for protein quality control

Takashi Kawamura[1], Takeshi Takahashi[1], Nobuhisa Watanabe*[2], Atsuo Suzuki[1], and Takashi Yamane[1]
[1]Department of Biotechnology and Biomaterial Chemistry,
[2]Synchrotron radiation research center, Nagoya University
Nagoya, Aichi, 464-8603, Japan

Introduction

Fbs2 is a component of SCF complex, E3 ubiquitin ligase working on the stage of the endoplasmic reticulum associated degradation (ERAD) system. In this system Fbs2 plays a role of sensor for detecting misfolded protein by interacting with chitobiose structure that is consensus motif of N-linked origosaccharide. Misfolded protein has N-linked origosaccharide with chitobiose structure exposed to solvent so that Fbs2 can interact with the motif. This interaction is necessary for ubiquitin ligation by SCF complex to targeted misfolded protein. The ubiquitinized protein is introduced into the ubiquitin-proteasome system for degradation. This elaborated system eliminates misfolded protein from our cells.

Fbs2 is a member of F-box protein that share F-box domain for interacting Skp1, an invariant component of SCF complex. Fbs2 has four sequence homologues in the human genome. They have varied affinity for N-linked origosaccharides. Among them, 3D structure of Fbs1 had been determined as complex with chitobiose and detailed interactions were revealed in atomic detail. But Fbs1 is expressed in specific cells such as a nerve cell and brain, while Fbs2 is expressed in a variety of cells in the human body. This suggests that Fbs2 is mainly involved in the ERAD pathway rather than Fbs1 that exist free from Skp1 and SCF complex.

Crystal of Fbs2 complex with Skp1 has large unit cell (c=405 Å). Careful data collection with fine oscillation angle and intensity data processing are required for the crystal structure determination.The Photon Factory Activity Report 2003 has adopted a new format for electronic publication of Users' Report. This document describes the requirements for submission of papers.

Data colection and structure determination

Crystallization condition of Fbs2-Skp1 complex was re-investigated for improvement of crystal quality. Initial crystal observed in the crystallization condition (containing PEG3350 and Na formate) was used for micro-seeding, and plate-like crystal clusters were obtained. Diffraction data were collected with the single plate-like crystal pealed from the cluster. Oscillation width of the frame was 0.3°. Diffraction data were reduced with HKL2000 and 3.5Å data were collected with over-90% completeness, which is a great progerss from previous study with only 60% completeness.

Space group of the crystal was re-investigated. It was previously assigned as P31, but corrected to P3121. The correction is attributed to improved completeness. Initial phases are determined by molecular replacement with Fbs2-Skp1 model partially constructed in previous study.

Table 1: Data collection statistics

Space group	$P3_121$
a (Å)	50.0
c (Å)	406.0
Resolution	50.0-3.50 (3.56-3.50)
Observed reflections	69869
Unique reflections	8232
Completeness	99.6 (99.1)
Redundancy	8.5 (6.7)
R_{sym} (%)	6.7 (56.3)

Model building of Fbs2-Skp1 complex was manually done with the program COOT. The electron density map of the Fbs2 is traceable, and particularly chitobiose binding domain of Fbs2 shows clear electron density with the aid of crystal packing. But Skp1 and interaction site between Skp1 and Fbs2 has poor electron density yet.

Results and Discussion

Fbs2-Skp1 complex structure was not fully determined yet, because of the poor crystal quality. But the structure of chitobiose-binding site is reliable. Chitobiose-binding site has Phe128, Phe232, and Trp233 that is comparable to Phe177, Tyr279, and Trp280 of Fbs1 for chitobiose binding. Conserved hydrophobic residues are thought to be that hydrophobic interactions are commonly shared between Fbs1 and Fbs2 for binding to sugar rings. Difference in this site is hydrophilic side chains observed in Fbs2 structure compared with Fbs1-chitobiose complex structure. S110 of Fbs2 comparable to D158 of Fbs1elinimates electronic charge of chitobiose-binding site. The side chain of the residue is interacted with chitobiose on the edge of the sugar ring.

*nobuhisa@nagoya-u.jp