Structural Basis of the γ-Lactone-ring formation in ascorbic acid biosynthesis by the the Senescence Marker Protein-30/Gluconolactonase

Ayaka Harada[1], Shingo Aizawa[2], Miki Senda[1], Naoki Maruyama[2], Akihito Ishigami[2], Toshiya Senda[1]

[1]*Photon Factory, Tsukuba 305-0801, Japan*
[2] *TMIG, 35-2 Sakaemachi, Itabashi-ku, Tokyo 173-0015, Japan*

1 Introduction

The senescence marker protein-30 (SMP30) exhibits gluconolactonase (GNL) activity. Biochemical and biological analyses revealed that SMP30/GNL catalyzes formation of the γ-lactone-ring of L-gulonate in the ascorbic acid biosynthesis pathway. The molecular basis of the γ-lactone formation, however, remains elusive due to the lack of structural information on SMP30/GNL in complex with its substrate. Here, we report the crystal structures of mouse SMP30/GNL and its complex with xylitol, a substrate analogue, and those with 1,5-anhydro-D-glucitol and D-glucose, product analogues.

2 Method

Diffraction data of mouse and human SMP30/GNL crystals were collected at 95K with an ADSC CCD detector using synchrotron radiation at BL-5A, BL-17A, and PF-AR NE-3A of Photon Factory (PF) in KEK (Tsukuba Japan). The diffraction data were processed and scaled using the programs XDS and XSCALE, respectively. The crystal structure of human SMP30/GNL was determined by the molecular replacement (MR) method with the program MOLREP in the CCP4 program suite using the earlier determined crystal structure of human SMP30/GNL (PDB ID: 3G4E) as a search model. Then, the structure of mouse SMP30/GNL was determined by the MR method using the structure of human SMP30/GNL. The crystal structures of mouse and human SMP30/GNL were refined using the program phenix.refine. Molecular models were built using the program COOT.

3 Results and Discussion

The overall structure of mouse SMP30/GNL was essentially the same as that of human SMP30/GNL. SMP30/GNL adopts a β-propeller structure, which is composed of six β-sheets each of which is formed with four β-strands. The superposition of mouse and human SMP30/GNL revealed that residues 120–129, which are located in a loop region connecting two β-strands, have different conformations between them. These residues are located at the top of the SMP30/GNL molecule, serving as a lid over the substrate-binding cavity of the SMP30/ GNL molecule.

Crystal structure of SMP30/GNL in complex with substrate/product homologues suggested that L-gulonate coordinates to the divalent metal ion. Then, Arg101, Asn103, and Glu121 seem to interact with the L-gulonate in the substrate-binding cavity, and the L-gulonate seems to bind to the substrate-binding cavity in a folded conformation. A manual modeling study suggested that L-gulonate in a folded conformation could be accommodated by the substrate-binding cavity of mouse SMP30/GNL. Then, nucleophilic attack of the hydroxyl group at C4 to the C1 atom may lead to the formation of the γ-lactone ring. In this step, Asp204 may serve as a catalytic base, which deprotonates OH(4) of the substrate, to facilitate the nucleophilic attack. In this catalytic reaction, hydroxyl groups of the substrate seem to be recognized by Arg101, Asn103, and Glu121, which are located at the one side of the inner surface of the substrate-binding cavity. These interactions seem to properly place the substrate in the active site and induce the substrate binding in a folded conformation.

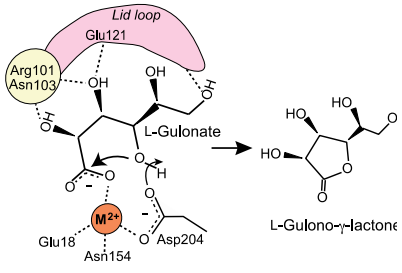

Fig. 1. Proposed catalytic reaction mechanism of mouse SMP30/GNL

[1] S. Aizawa *et al*., *PLOS ONE*8(1): e53706. doi:10.1371/journal.pone.0053706