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X-ray structure of *Oryza sativa* hexokinase 6 in complex with β-D-glucose

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1 Introduction

Some hexokinases are found in *Oryza sativa*, and one of which, hexokinase 6 (OsHXK6) shows enzymatic activity not only to D-glucose but also to D-allose. D-Allose is known as one of rare sugars which exist in a small amount in nature. Gibberellin is a plant hormone that is involved in growth regulation. So far, D-allose is reported to inhibit the gibberellin-dependent responses such as elongation of rice leaves, and induce resistance to a phytopathogenic bacterium *Xanthomonas oryzae* pv. *oryzae* including induction of lesion mimic formation and pathogenesis-related protein gene expression in rice [1-4]. D-Allose suppresses expressions of gibberellin-responsive genes through hexokinase-dependent pathway.

In this study, we determined crystal structure of OsHXK6 in complex with β-D-glucose and AMPPNP (ATP analogue) at 2.84 Å resolution.

2 Experiment

The recombinant OsHXK6 was used for crystallization. After initial screening and optimization using the concentrated protein solution (4.3 mg/mL), crystals were obtained by mixing the protein solution (0.8 μL) and the same volume of reservoir solution (0.1 M MOPSO, 0.1 M Bis-Tris pH 6.5, 10 mM Spermine tetrahydrochloride, 10 mM Spermidine trihydrochloride, 10 mM 1,4-diaminodutane dihydrochloride, 10 mM D,L-Ornithine monohydrochloride, 20% (w/v) 1,5- pentanediol, 10% (w/v) PEG8000) including 5 mM D-glucose, AMPPNP, $MgCl_2$, against 50 μL of the reservoir solution by the sitting-drop method at 293 K. X-ray diffraction data were collected on the PF BL-5A in the KEK, and processed using the programs XDS and the CCP4 program suite. Initial phase was determined by molecular replacement using the structure of hexokinase 1 from *Arabidopsis thaliana* (AtHXK1, PDB code: 4QS7) as a probe model.

3 Results and Discussion

OsHXK6 was crystallized in space group $P3_221$, ($a = b = 131.60$ Å, $c = 188.92$ Å) and the structure was refined to R-factor of 0.206 at 2.84 Å resolution. The overall structure of OsHXK6 with bound β-D-glucose, AMPPNP and Mg is similar to that of AtHXK1 in β-D-glucose bound form (4QS7, closed form) with 68 % identity, 1.2 Å rmsd, human glucokinase in complex with α-D-glucose and a small molecule activator (4IXC, 35 %, 1.9 Å), hexokinase from *Schistosoma mansoni* complexed with α-D-glucose (1BDG, 39 %, 1.8 Å) and AtHXK1 in ligand free form (4QS8, open form, 67 %, 3.2 Å).

Fig. 1 shows the structure of OsHXK6 in complex with β-D-glucose, AMPPNP and Mg, and the active site structure with bound β-D-glucose. It showed the closed form by the approach of the region colored in pale yellow (His102-Val236) including Thr202 and Lys203, to bind β-D-glucose.

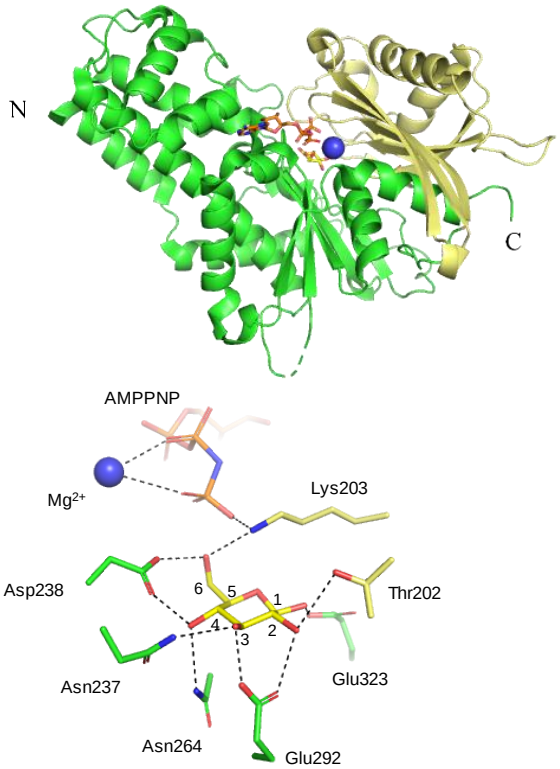

Fig. 1: The structure of OsHXK6 in complex with β-D-glucose, AMPPNP and Mg (deposited as 5ZQT, closed form), and the active site structure with bound β-D-glucose.

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