Crystallography

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Structure of salt-tolerant glutaminase from *Micrococcus luteus* K-3 in the presence and absence of tris and L-glutamate

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Introduction

Glutaminase (EC 3.5.1.2) hydrolyses L-glutamine to produce L-glutamate. L-Glutamate production is important for food process industry, because L-glutamate is a savourous amino acid. Thus, glutaminase activity is necessary for various fermentations such as the soy sauce fermentation. However, most of glutaminases are inhibited by high salt concentrations in soy sauce. Salt-tolerant glutaminase is required for brewing high quality soy sauce with a high glutamic acid concentration [1]. Glutaminase of *Micrococcus luteus* K-3 (*Micrococcus* glutaminase) is a salt-tolerant enzyme that shows 40 % residual activity even in the presence of 3 M NaCl. In this study, the structure of *Micrococcus* glutaminase in the presence and the absence of its product L-glutamic acid and its activator tris was determined.

Methods and Results

Crystalization and Data Collection

Micrococcus glutamianse was crystallized in the presence and the absence of 0.2 M L-glutamic acid and 0.3 M tris. The crystals of *Micrococcus* glutamiasne were obtained by the hanging drop vapor-diffusion method at 20˚C. The structure of *Micrococcus* glutamianse was determined by the molecular replacement method using the fragment of *Micrococcus* glutaminase as a search model (PDB code, 2DFW [2]). The model refinement was performed using the program CNS 1.2, and the model was fitted manually using the O program.

Figure 1 shows the structures of the physiological dimer of *Mocirococcus* glutaminase in the four conditions. The obtained structure (no additive form referred to as N) has 36 disordered regions at residues from 355 to 376 and 447-456. By the addition of 0.3 M tris that activates about 6 fold, the structure (tris form referred to as T) has 8 disordered residues. In the presence of its product L-glutamate, the density for L-glutamate is very weak in the structure (L-glutamate form referred to as G) and it has 19 disordered residues (353-359, 397-402 and 449-456 aa). By the addition of both tris and L-glutamate (tris and L-glutamate form referred to as TG), the structure has 6 disordered amino acid residues. The TG structure revealed that its product L-glutamic acid locates in the deep cleft of the N-terminal domain (1-305 aa).

References

[1] R. Nandakumar et al., J. Mol. Catal. B 23, 87-100 (2003).
[2] K. Yoshimune et al., Biochem. Biophys. Res. Commun. 346, 1118 (2006).
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Figure 1. Stereo view of the physiological dimers. The N (yellow), T (blue), G (red), and TG (black) structures and the other molecules in the physiological dimer are shown in lemon (N), light blue (T), pink (G), and grey (TG). L-Glutamates are shown by spheres (magenta).