Biological Science

6A/2009G012

Crystal structure of salt-labile glutaminase from *Bacillus subtilis* in the presence of 4.3 M NaCl

Kazuaki YOSHIMUNE*[1]
Bioproduction Research Institute, AIST, Sapporo, Hokkaido 062-8517, Japan

Introduction

Glutaminase (EC 3.5.1.2) hydrolyzes glutamine to produce glutamate that is a savourous amino acid. Therefore, glutaminase activity is important for food process industry such as the soy sauce fermentation. Because most of usual glutaminases are inhibited by high salt concentrations in soy sauce, salt-tolerant glutaminase is required for brewing high quality soy sauce. To increase the salt-tolerance of salt-labile glutaminase, understanding of the inhibitory mechanism of salt-labile enzyme is also necessary as well as understanding of the salt-tolerant mechanism of the salt-tolerant enzyme. Our previous study revealed that the structure of salt-tolerant glutaminase from *Micrococcus luteus* (Mglu) is similar to that of salt-labile glutaminase from *Bacillus subtilis* (*Bacillus* glutaminase) [1, 2]. The effect of NaCl on the structure of salt-labile enzyme has not been investigated because of a defect in the structural information in the presence of high concentration of NaCl. Here, the structure of *Bacillus* glutaminase in the presence of 4.3 M NaCl was determined, and the structure was compared to that of the previously determined structure in the absence of NaCl.

Methods and Results

Crystallization and Data Collection

Bacillus glutaminase was crystallized in the presence of 4.3 M NaCl, and the crystals of *Bacillus* glutaminase were obtained by the microbatch method under paraffin oil at 20 °C. The structure of *Bacillus* glutaminase was determined by the molecular replacement method using the structure in the absence of NaCl as a search model (PDB code, 1MKI [3]). The model refinement was performed using the program CNS 1.2, and the model was fitted manually using the O program.

Determination of structure

Bacillus glutaminase was crystallized in the presence of 4.3 M NaCl under new crystallization conditions that included the use of NaCl as a precipitant (previously, PEG 1,500 was used [3]). There is the physiological dimmer in the asymmetric unit of $P2_12_12$ that is the same as that of the previously determined structure in the absence of NaCl [3], and the structures of both monomers are similar. The determined structure of *Bacillus* glutaminase in the presence of 4.3 M NaCl (PDB code, 3AGF) shows no significant structural difference with the published structure in the absence of NaCl (1MKI [3]) as shown in Figure 1. Further structural analysis of the structural change during the catalysis in the presence of NaCl may reveal the inhibitory mechanism of NaCl.

References

[1] K. Yoshimune et al., Biochem Biophys Res Commun 346, 1118 (2006)
[2] K. Yoshimune et al., FEBS J 277, 738 (2010)
[3] G. Brown et al., Biochemistry 47, 5724 (2008)

* k.yoshimune@aist.go.jp

Figure 1. Effect of 4.3 M NaCl on the backbone atoms of *Bacillus* glutaminase. The backbone atoms of the structures of *Bacillus* glutaminase in the presence and absence of NaCl are aligned. The averages with standard deviations of the displacement values are shown in the figure.