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Determination of overall structure of salt-tolerant glutaminase from *Micrococcus luteus* K-3

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Introduction

Glutaminase (EC 3.5.1.2) catalyzes the hydrolytic degradation of L-glutamine to L-glutamic acid. 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, and belongs to the serine-dependent β-lactamases family. *Micrococcus* glutaminase shows 32 and 29 % similarity with glutaminases of *Escherichia coli* (Ybas) and *Bacillus subtilis* (Ybgj), respectively [1,2]. An optimum pH of Ybas is 4.0, that is 4 units lower than the optimum pHs of *Micrococcus* glutaminase and Ybgj, and Ybgj is salt labile protein. To investigate mechanism to characterize the optimum conditions of these glutaminases, their crystal structures were necessary. The fragment structure of *Micrococcus* glutamianse (42 kDa) has been determined [2], its overall structure (48 kDa) is unknown. In this study, the overall structure of *Micrococcus* glutaminase with its product L-glutamic acid was determined.

Methods and Results

Crystalization and Data Collection

The recombinant *Micrococcus* glutamianse was purified as previously described [2], and was crystallied in the presence of its product L-glutamic acid. The crystals of *Micrococcus* glutamiasne were obtained by the hanging drop vapor-diffusion method at 20˚C. The crystals belong to the space group C2 with unit cell dimensions of a=118, b=141, and c=74Å. 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.

The structure revealed that its product L-glutamic acid locates in the deep cleft of N-terminal domain (Fig. 1) and its overall structure (Fig.2). This is the first report that shows the structure of the disordered region (355-404aa). Based on the results of this observation and site-directed mutagenesis previously reported [3], the cleft is considered to be its active site. Brown et al have shown the covalent complex structure of *B. subtilis* glutaminase with its inhibitor 6-ziazo-5-oxo-L-norleucine (DON), and also suggest the active site [1] that is homologous to the putative active site of *Micrococcus* glutaminase.

Further work is required to further characterize the salt-tolerant mechanisms of *Micrococcus* glutaminase.

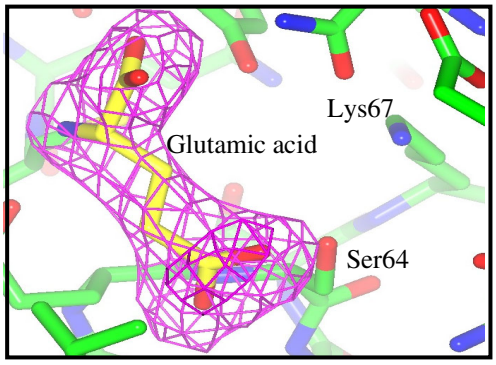

Figure 1. The catalytic site of *Micrococcus* glutaminase with L-glutamic acid. Magenta density represents the F_o-F_c map contoured at 3.0σ. Protein residues (green carbon atoms) and L-glutamic acid (yellow carbon atoms) are shown as a stick representation.

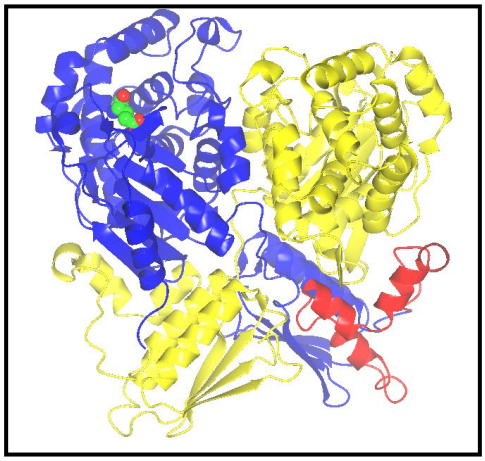

Figure 2. Overall structure of the dimmers. Protein subunits are shown in blue and red, and yellow. The amino acid residues (355-404) determined by this experiment is shown in red, and L-glutamic acid is shown as green carbon atoms.

References

[1] G. Brown et al., Biochemistry. 47, 5724 (2008).
[2] K. Yoshimune et al., Biochem. Biophys. Res. Commun. 346, 1118 (2006).
[3] S. Yano et al., J. Biosci. Bioeng. 102, 362 (2006).

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