Structural Studies of Human Glutaminyl Cyclase by X-ray Crystallography

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Glutaminyl cyclase (QC) has been identified to exist in a number of animals, plants and bacteria. The papaya QC was proven to have an exceptional resistance to protease-degradation as well as chemical, thermal and acid denaturation *in vitro*. Interestingly, though the QCs from plants and mammals are usually similar in size, little or no sequence homology between them have been observed thus far. A highly conserved primary structure of various QCs, however, was reported among different mammalian species. Nevertheless, the detailed catalysis mechanism and the three-dimensional structure of this enzyme are still poorly understood and remain to be elucidated, in spite of the proposition that it might proceed through acid/base promotion of the direct cyclization of the glutaminyl residue via a five-membered cyclic intermediate.

In the current project, we would like to prepare the recombinant QCs (from a variety of source including human, yeast or papaya) in bacteria or yeast expression system to a large-scale. These QCs will be crystallized and subjected to X-ray diffraction analysis in order to obtain their crystal structures by the MAD (multiplewavelrngth-anomalous diffraction) method. Based on the elucidated structure(s), we plan to make efforts on the designs of a stable and effective QC for the purpose of generating more physiologically important and *p*Glu-containig proteins and peptides. In addition, we attempt to explain the catalysis mechanism of QC, based on the solved QC structures.

We already got the crystals of human glutaminyl cyclase and its Se-Met derivative. In the present experiments, we successfully obtained the fluorescence spectra of the crystals of Se-substituted QC (Figure 1), which are useful for us to select the precise wavelengths for data collections. We have collected the enough data with high resolution in order to solve the QC structure by the MAD or SAD method. We are very grateful to NSRRC for supporting enough x-ray beam time. We also thank Dr. Yu-San Huang for assistance of data collections.

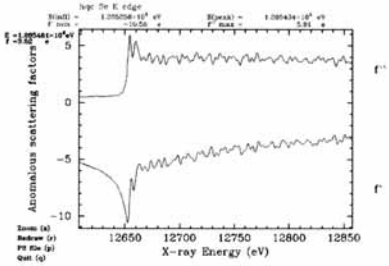

Figure 1

Structural Determination of the Guanine Deaminase from *Bacillus subtilis*

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With a rapidly increasing number of sequences and structures, one major challenge in the post-genomic era is to understand how nature utilizes one structural fold to serve many diverse protein functions, so that *in vitro* methods can be used to generate "new" enzymes for medical and industrial applications. Structure determination and comparison of homologous proteins in a structural superfamily provides an efficient path to understand the evolution history of structural plasticity and functional versatility.

Purine/pyrimidine bases and nucleotides taken up from the environment or formed intracellularly serve not only catabolic purposes as nitrogen and carbon sources but also anabolic purposes in the nucleotide synthesis. A deamination step is the first and committed step in their degradation and salvage pathways. Therefore, the purine/pyrimidine deaminases play key roles in the nucleotide metabolism and then become attractive candidates for antibacterial and anticancer therapy.

Interestingly, most of purine/pyrimidine deaminases belong to the cytidine deaminase (CDA) and TIM barrel metallohydrolase superfamilies. Our analysis reveals that CDAs, dCMP deaminases, fungal cytosine deaminase (CD), *B. subtilis* guanine deaminase (GD), riboflavin biosynthesis protein RibD and the catalytic domains of RNA editing enzymes comprise the first superfamily. On the other hand, adenine deaminases, adenosine deaminases, AMP deaminases, bacterial CDs, and most GDs belong to the latter one. Therefore, these two superfamilies provide an elegant example of convergent evolution, where starting from unrelated ancestral proteins, the same reactions are achieved through distinctly different active sites.

In order to understand the evolutionary history of the CDA fold and its substrate specificity, here we would like to determine the crystals structure of *Bacillus subtilis* GD. The Se-MAD and native data sets have been collected, and the structure has been solved (Table 1; Fig. 1). Structural refinement is under investigation.

Table 1. Data-collection statistics

	Wavelength	Resolution	Reflections	Completeness	Rmerge
GD-Se	0.9798 Å	1.51 Å	46806	95.3 %	7.3 %
GD-Se	0.9799 Å	1.55 Å	42936	94.5 %	8.7 %
GD-Se	0.9571 Å	2.0 Å	17470	81.4 %	9.9 %
GD	1.0 Å	1.45 Å	53874	97.6 %	3.9 %
GDI	0.9798 Å	1.15 Å	99896	93.9 %	5.2 %

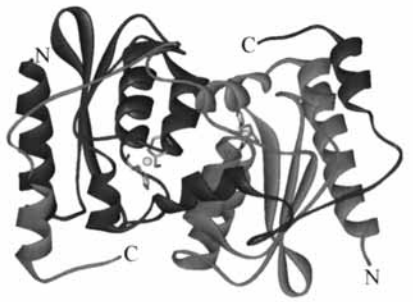

Fig. 1. The dimeric structure of *B. subtilis* GD. Unexpectedly, this is the first identified domain-swapped protein in the CDA superfamily.