Crystal Structures of γ-Glutamyltranspeptidase from *Escherichia coli* and its Reaction Intermediate

γ-Glutamyltranspeptidase (GGT) is an extracellular enzyme that plays a key role in the metabolism of γ-glutamyl compounds including glutathione (GSH) [1]. GGT catalyzes the first step of the degradation of extracellular GSH into constituent amino acids, which are then transported into the cell and used as cysteine and nitrogen sources in *Escherichia coli*, yeast, and mammalian cells [2]. In mammals, GSH/xenobiotic conjugates are cleaved by GGT and these products are metabolized, leading to the excretion of mercapturic acids into the bile and urine. Clinically, GGT is widely used as a marker in blood tests; high level of GGT activity in the blood is indicative of hepatic- and biliary-tract-associated diseases.

GGT is a heterodimer comprising a large (L) subunit and a small (S) subunit generated from a precursor protein by posttranslational processing [3]. GGT catalyzes the hydrolysis of the γ-glutamyl linkage of its substrates to yield glutamate and the transfer of the γ-glutamyl group to other amino acids and peptides (Fig. 1). We determined the crystal structure of *E. coli* GGT, which shares a similar primary structure and enzymatic characteristics with mammalian GGTs, at a resolution of 1.95 Å (R = 20.7%, R_{free} = 23.1%) [4]. Furthermore, we analyzed the course of its enzymatic reaction by X-ray crystallography, in which GGT crystals that were flash-cooled after soaking in GSH solution for various soaking times were used. We also determined the structure of GGT in complex with L-glutamate (GGT-Glu), the product of hydrolysis. All diffraction data were collected at beamline **BL41XU**.

The present analysis first revealed the tertiary structure of GGT; GGT exhibits the stacked αββα fold seen in the members of N-terminal nucleophile hydrolase superfamily (Fig. 2(a)). The β-strands in the L and S subunits form the two central β-sheets, which are flanked by α-helices. Thr-391, the N-terminus of the S subunit generated by posttranslational processing, is the active residue of catalysis. Thr-391 was located in the bottom of the deep groove, from which the pocket for γ-glutamyl moiety binding follows (Fig. 2(b)). The pocket was shielded from the solvent by the surface loop.

A Fourier map for the crystal soaked in the GSH solution for 10 sec showed the density of the γ-glutamyl moiety in the substrate binding pocket (Fig. 3(a)). Remarkably, the Oγ atom of Thr-391 was covalently bonded to the carbonyl carbon of the γ-glutamyl moiety, illustrating that the γ-glutamyl-enzyme intermediate was trapped. This structure represents the state generated by the carbonyl carbon at the γ-position of GSH being attacked by Thr-391 Oγ, which is followed by the release of the Cys-Gly moiety of GSH. Moreover, a water molecule (labeled W2 in Fig. 3(a)) was seen on the carbonyl carbon of the γ-glutamyl-Thr-391-Oγ bond in the intermediate that is to be hydrolyzed. When the GGT crystals were soaked for 1 min or more, the electron density between L-glutamate and Thr-391 was resolved, showing that the hydrolysis of the γ-glutamyl-enzyme intermediate occurred in the crystalline state (Fig. 3(b)). Although the conformation beyond the Cβ in the γ-glutamyl moiety differs from that in glutamate, the binding manner of the α-amino and α-carboxyl groups of the γ-glutamyl moiety was identical to that of L-glutamate.

Extensive site-directed mutagenesis studies of

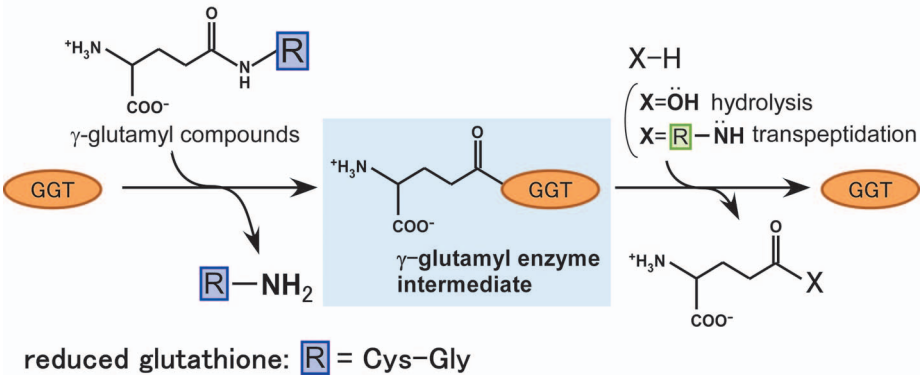

Fig. 1. Reactions catalyzed by GGT.

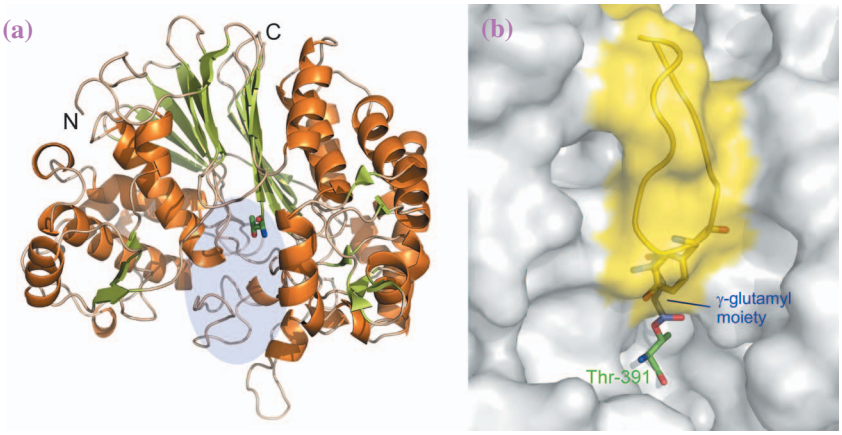

Fig. 2. **(a)** Ribbon drawing of *E. coli* GGT. α-helices are colored brown and β-strands are colored green. The active residue, Thr-391, is shown as a stick model and the substrate binding pocket is shaded. **(b)** Surface drawing around substrate binding pocket. The ribbon (yellow) represents the loop that shields the pocket from solvent.

human GGT identified the residues essential for GGT activity (i.e., Arg-114, Asp-433, Ser-462, and Ser-463 in *E. coli* GGT). These are all involved in the binding of the α-amino and α-carboxyl groups of the substrate. Although the involvement of Glu-108 of human GGT (Glu-115 in *E. coli* GGT) in acceptor binding was shown, Glu-115 is buried inside GGT, where it is hydrogen-bonded to Arg-114, the residue responsible for α-carboxyl-group recognition. These residues are all highly conserved in the GGT amino acid sequences of many species. Future studies on other GGTs including human GGT can be carried out on the basis of the structure of *E. coli* GGT.

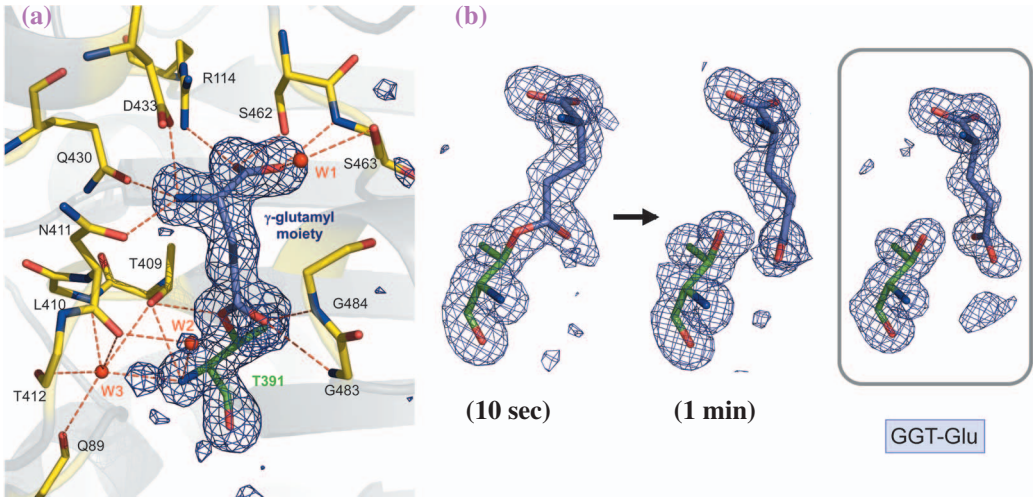

Fig. 3. **(a)** Electron densities of γ-glutamyl moiety and Thr-391 in γ-glutamyl-enzyme intermediate. Ball and stick models are overlaid on the map. **(b)** Hydrolysis of γ-glutamyl-enzyme intermediate. Stick models of γ-glutamyl moiety, L-glutamate and Thr-391 are overlaid on the electron densities. The bond between the γ-glutamyl moiety and Thr-391 in the intermediate was hydrolyzed in 1 min. The map on the right shows the structure of the GGT-Glu complex.

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