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A Preliminary Description of the Crystal Structure of γ-Glutamyltranspeptidase from *E. coli* K-12

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The structure of GGT [EC 2.3.2.2] from *E. coli* K-12 was studied at 3 Å resolution by X-ray crystallography. Initial protein phases were calculated using two kinds of Pb^{2+} derivatives. The phases were refined by non-crystallographic 2-fold symmetry electron density averaging combined with solvent flattening and histogram matching. The GGT molecule has overall dimensions of 60×50×40 Å. There are two antiparallel β-pleated sheets consisting of 6 and 7 β-strands. The two β-sheets form a wall-like structure. Twelve short α-helices were detected, of which the maximum length appears to be four helix turns.

Key words: crystal structure analysis, density modification, γ-glutamyltranspeptidase.

γ-Glutamyltranspeptidase [GGT, EC 2.3.2.2], an enzyme of major importance in glutathione metabolism, catalyzes the hydrolysis of γ-glutamyl compounds such as glutathione, and the transfer of glutamyl moieties to amino acid and peptides (*1*). This enzyme is widely distributed in living organisms (*2*) and has some homologous parts in its primary sequence (*3, 4*). However, none of its crystal structure has been solved.

GGT from *Escherichia coli* K-12 is a periplasmic enzyme with a molecular weight of 59,208 Da, and is composed of one large and one small subunit. The large subunit consists of 365 amino acid residues and the small one consists of 190 amino acid residues. The amino acid sequence has been deduced from the nucleotide sequence of cloned DNA (*4*). GGT of *E. coli* K-12 is the only enzyme which has been crystallized (*5*) and for which crystallographic properties have been reported (*6*). We describe here phase improvement by density modification techniques, the shape of a GGT molecule and the secondary structures.

GGT was purified from the periplasmic fraction of a recombinant strain of *E. coli* K-12 (SH642) by lysozyme treatment, ammonium sulfate precipitation and chromatofocusing (*7*). Native crystals were obtained from 15% PEG6000 in 20 mM acetate buffer, pH 5.2. The crystals belong to the space group, $P2_12_12_1$, with cell dimensions of $a=128.1$, $b=129,9$, and $c=79.2$ Å, and contain two GGT molecules in an asymmetric unit.

Two lead derivatives, designated as Pb1 and Pb2, were respectively prepared by soaking native crystals in a buffer solution containing 2.5 mM $Pb(CH_3COO)_2$ for 4 days, and in the buffer solution containing 5 mM $Pb(CH_3COO)_2$ and 10 mM ICH_2COOH for 3 days. Since upon inspection of the cell dimensions, the derivatives thus obtained turned out not to be isomorphous with the native crystals, "parent crystals" were newly prepared by immersing the native crystals in the buffer solution containing 5 mM ICH_2COOH for four days (Table I).

X-ray diffraction data were collected with a Weissenberg camera for macromolecular crystallography (*8*) at the beam line BL6A in the Photon Factory synchrotron radiation source, Tsukuba. The operation condition for the Weissenberg camera with an imaging plate (IP) cassette of 430 mm radius was typically a 4.5° oscillation for each with a coupling constant of 1.5°/mm. The intensity data for parent crystals were collected using a wavelength of 1.0 Å while those for Pb derivatives were collected using a wavelength of 0.92 Å in order to optimize the anomalous dispersion effect. The intensity data recorded with the BASIII type IP were digitized with a Fuji-BA100 IP scanner. The data were automatically indexed (*9*), integrated, and corrected for the Lorenz and polarization effects with the program, "WEIS" (*10*), and scaled by means of the program system, "PROTEIN" (*11*). The data are shown in Table I. All other calculations described below were performed using programs contained in the CCP4 suite (*12*).

To determine the heavy atom positions, three-dimensional isomorphous and anomalous difference Patterson maps were calculated for the Pb1 and Pb2 derivatives at 5 Å resolution. One major site was found in the three Harker sections of the map and the atomic parameters were refined with the program, MLPHARE. The initial phases were calculated by the single isomorphous replacement method

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Abbreviations: GGT, γ-glutamyltranspeptidase [EC 2.3.2.2]; IP, imaging plate; NCS, non-crystallographic symmetry.

incorporating anomalous dispersion effects (SIRA), and additional lead sites were found through the difference Fourier maps calculated with these phases. Finally two main sites and two minor sites were determined for the Pb1 derivative, and three main sites and one minor site were determined for the Pb2 derivative. The positional parameters nearly coincided with each other for the two derivatives. However, the relative occupancies of the sites were different in the two derivatives. At the stage of final phase calculation, the mean figures of merit were 0.586 and 0.398 for 2,122 centric and 18,922 acentric reflections, respectively. In order to refine the phases, we used the program, SQUASH, employing a combination of histogram matching and solvent flattening techniques.

Since one asymmetric unit contains two GGT molecules, self rotation function maps were calculated using the programs, ALMN and POLARRFN. Both indicated the existence of a local 2-fold axis in the direction defined by the Euler angles of $\omega = 39.9°$, $\psi = 90°$, and $\kappa = 18°$. Electron density averaging using a non-crystallographic symmetry (NCS) 2-fold axis was performed in order to further improve the phases. A protein mask corresponding to the shape of one GGT molecule was initially made in the previous electron density maps at 3.5 Å resolution using the programs, MAPMAN and MAMA. The rotational matrix and translation vectors (NCS parameters) were determined by rotating and translating the electron density within the mask in the initial electron density map at 3.5 Å resolution with an approximate grid spacing of 1.0 Å. These parameters were further refined with the program, IMP. The refined rotational matrix was almost the same as that obtained from the self-rotation function. The final correla-

TABLE I. **Cell dimensions and statistics of intensity data up to 3 Å resolution.** For the preparation of Parent, Pb1 and Pb2 crystals, see the text.

Crystal dimensions	Parent	Pb1	Pb2
Cell dimensions			
a (Å)	128.2	127.8	127.9
b (Å)	130.6	129.9	129.6
c (Å)	77.8	77.0	77.0
Reflections (>1 sigma)			
Total	79,361	83,634	75,725
Unique	21,820	22,673	22,368
Completeness (%)	81.3	85.7	84.9
R (merge)	0.0829	0.0641	0.0835

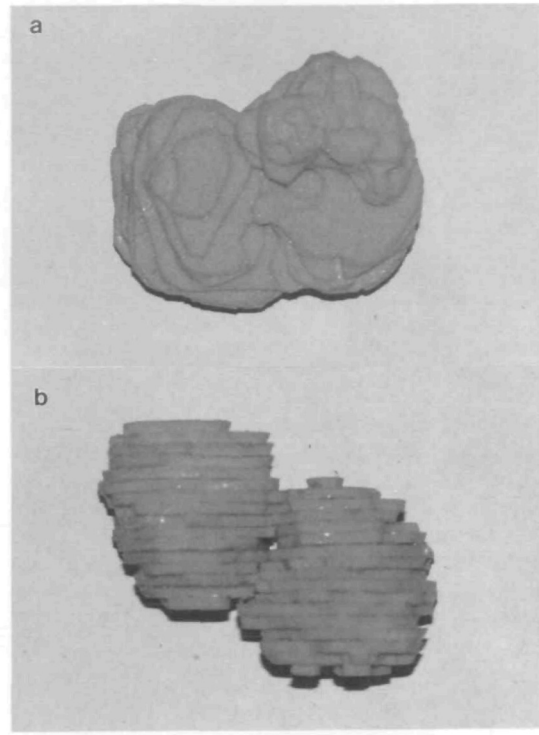

Fig. 2. **A molecular model of two GGT molecules (one dimer) in an asymmetric unit, which was made on the basis of the final electron density maps at 3.5 Å resolution.** The size of the model is about $80 \times 63 \times 50$ Å^3. (a) The model viewed from the *a*-axis direction. (b) The model viewed from the *c*-axis direction. The NCS twofold axis penetrates a hole in the contact surface of the two molecules.

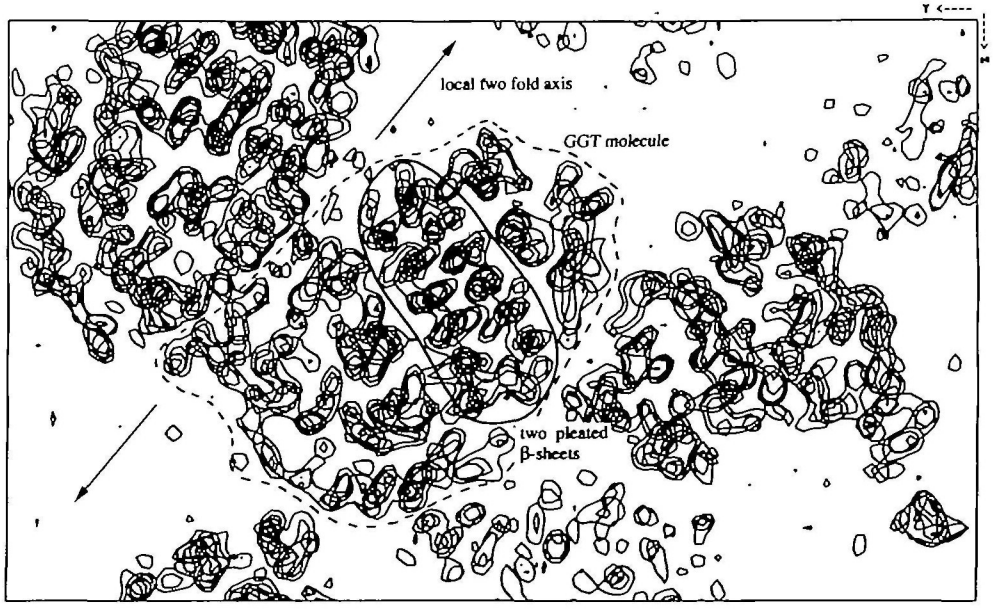

Fig. 1. **Composite electron density maps at 15-3.5 Å resolution from 25/80 to 29/80 sections along the *a*-axis direction.** The maps were calculated with phases refined by NCS averaging combined with solvent flattening and histogram matching. A GGT molecule is surrounded by a dotted line. Two β-sheets are surrounded by a solid line at the central part of the molecule. The position of the NCS twofold axis is indicated by two arrows.

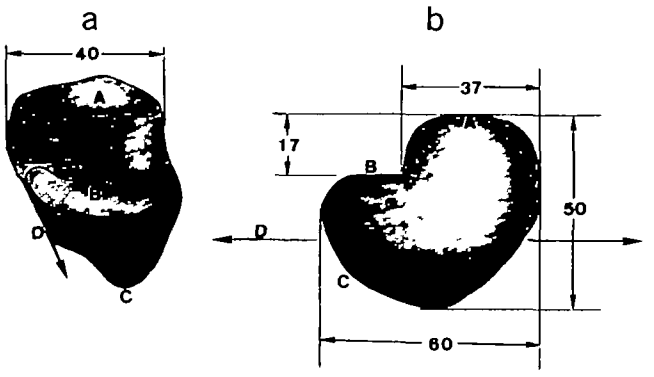

Fig. 3. **Sketch drawings of a GGT molecule viewed from nearly [101] direction (3a) and [010] direction (3b).** The secondary structures present in part A are mainly α-helices and those in part B are mainly two anti-parallel β-sheets surrounded by α-helices. The approximate dimensions are given in Angstrom units. The molecule can be conveniently divided into three domains, A, B, and C. The direction of the NCS twofold axis is indicated by the pair of arrows designated as D.

tion coefficient between the NCS-related protein electron densities reached up to 44%.

NCS averaging combined with solvent flattening and histogram matching was performed using the program, DM. The refinement was undertaken from 4.5 Å resolution using the previously refined phases and the resolution range was gradually extended up to 3 Å resolution. The final free R factor and electron density correlation coefficient between the NCS-related molecules for 21,478 reflections were 0.33 and 0.83, respectively. The resultant electron density map was apparently improved, and the boundary between the two GGT molecules related by the NCS twofold axis in an asymmetric unit was clearly revealed in the maps. Figure 1 shows the electron density distribution of the two GGT molecules, where β-sheets and α-helices are revealed clearly. The model of two GGT molecules (Fig. 2, a and b) in an asymmetric unit was made on the basis of the final electron density map (Fig. 1). The NCS twofold axis penetrates through the hole at the center of the model shown in Fig. 2b. The overall dimensions of the dimeric model are about $80\times63\times50$ Å^3. Although the mode of contact between the two molecules is rather tight, it was not difficult to separate the molecules from each other. The projections of a GGT molecule viewed from nearly [101] and [010] directions are shown in Fig. 3, a and b, respectively. The approximate dimensions shown in Fig. 3 are given in Angstrom units. In Fig. 3a the contact region of two molecules is located along the local twofold axis indicated by the arrow designated as D in Fig. 3. The overall shape of the GGT molecule is a deformed ellipsoid. The bottom side of the molecule, denoted by B and C in Fig. 3a, is a deformed ellipsoid, half of the top side (B) being nearly a plane and the other half of the top side (A) being approximately cylindrical. At the center of the bottom side (under B) there are two antiparallel pleated β sheets, each of which has seven and six strands, and these two β-sheets form a large wall, as shown in Fig. 1. The wall is surrounded by helices. In the core of the cylindrical part (A), there is a short length of anti-parallel β-sheet which is surrounded by four helices. This structure suggests that the top side is more flexible than the bottom side. The total number of helices is twelve and the maximum number of helix turns is four. Thus, 30 and 20% amino acid residues contribute to form the α-helix and β-sheets, respectively. There is a cavity which might be an active site near the contact region of the two molecules, as shown in Fig. 3a. Detailed crystal structure analysis is now in progress.

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