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New Crystal Forms of a μ-Class Glutathione S-Transferase from Rat Liver

BY JIAN-HUA FU AND JOHN ROSE

Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260, *USA*

MING F. TAM

Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 11529, *Taiwan*

AND BI-CHENG WANG*

Departments of Crystallography and Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, *USA*

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Abstract

Two new crystal forms of isoenzyme 3-3 of rat liver glutathione S-transferase (GST 3-3) have been obtained. They were grown under essentially the same crystallization conditions as those reported for the *C*2 crystal form [Fu, Rose, Chung, Tam & Wang (1991). *Acta Cryst.* B**47**, 813–814]. The new crystals belong to space group $P2_1$ with one form having cell dimensions $a = 101.6$, $b = 69.5$, $c = 81.4$ Å and $\beta = 113.6°$, and the other form having cell parameters $a = 97.4$, $b = 81.1$, $c = 69.4$ Å and $\beta = 109.2°$. These new crystals diffract to at least 2.5 Å resolution. The molecular packing arrangements in these $P2_1$ crystals have been found by molecular replacement studies.

Introduction

The glutathione S-transferases (GSTs, E.C. 2.5.1.18) are a family of dimeric proteins that catalyze the nucleophilic attachment of glutathione (GSH) to a wide variety of electrophilic alkylating agents. They form an important class of enzymes which are responsible for cell detoxification of endogenous substances and chemical carcinogens such as xenobiotic compounds. GSTs have also been shown to play key roles in the reduction of organic hydroperoxides, isomerization of prostaglandins and binding of non-substrate hydrophobic ligands such as bile acids, bilirubin, various drugs and thyroid hormones. In addition, there is evidence indicating the involvement of GSTs in the development of cell resistance to electrophilic anticancer drugs (Hayes & Wolf, 1988; Waxman, 1990). Recent reviews on the structures and biological functions of GSTs have been given by Mannervik & Danielson (1988), Pickett & Lu (1989) and Armstrong (1991).

The mammalian cytosolic glutathione transferases are composed of subunits of molecular masses of about 25 000 Da. The various subunits are coded by four distinct gene families, namely α, μ, π (Mannervik, 1985) and θ (Meyer, Coles, Pemble, Gilmore, Fraser & Ketterer, 1991). Subunits within the same gene family share a sequence homology of approximately 60–85%, but sequence identities across gene families are significantly less, in the range 20–30%. Within a gene family, homodimers as well as heterodimers exist. However, there has been no observation of the formation of heterodimers between different gene classes. Over the past few years, significant progress has been made in solving the three-dimensional structures of GSTs of different gene families. Among the many GST crystals reported (Sesay, Ammon & Armstrong, 1987; Schaffer, Gallay & Ladenstein, 1988; Cowan, Bergfors, Jones, Tibbelin, Olin, Board & Mannervik, 1989; Parker, Lo Bello & Federici, 1990; Dirr, Mann, Huber, Ladenstein & Reinemer, 1991; Fu *et al.*, 1991), four structure determinations, two for class π GST, one for class μ GST and one for class α GST have been completed (Reinemer, Dirr, Ladenstein, Schaffer, Gallay & Huber, 1991; Reinemer, Dirr, Ladenstein, Huber, Lo Bello, Federici & Parker, 1992; Ji, Zhang, Armstrong & Gilliland, 1992; Sinning, Kleywegt, Cowan, Reinmer, Dirr, Huber, Gilliland, Armstrong, Ji, Board, Olin, Mannervik & Jones, 1993). From the four structures, a striking feature common to these enzymes emerges in that the hydroxyl group of a highly conserved Tyr residue on the small domain is in close proximity to the sulfur of the bound substrate or substrate analog. It has been suggested that this key interaction allows the enzyme to activate the sulfhydryl group of GSH by effectively lowering its pK_a value.

While a similar overall folding pattern is observed for the various GST structures, there are conforma-

* Author for correspondence.

Table 1. *Data collection and processing*

Crystal form	No. of measured reflections	No. of unique reflections	d_{min} (Å)	R_{sym}*	$\langle I/\sigma(I)\rangle$	Completeness† Resolution range (Å)	%	Resolution range (Å)	%
A (*C*2)‡	72805	36151	2.49	0.071	19.8	−>2.6	97	2.6–2.49	40
B ($P2_1$)‡	96085	38166	2.46	0.051	27.3	−>2.6	98	2.6–2.46	45
C ($P2_1$)‡	66482	28925	2.68	0.076	16.0	−>2.8	99	2.8–2.68	53

* $R_{sym} = \sum_h \sum_i^N |F_i - \langle F\rangle| / \sum_h \sum_i^N F_i$, where F_i is the ith measurement of reflection h and $\langle F\rangle$ is the mean value of the N equivalent reflections of the index h.

† Completeness is the ratio of the total number of measured to that of the predicted unique reflections for the given resolution range.

‡ X-ray data were processed in space group *P*2 for the purpose of evaluating the systematic absences.

tional differences among the different GST classes (Ji *et al.*, 1992), as well as among GSTs from different species within a class (Reinemer *et al.*, 1992). Molecules of GST within the crystal, as observed in solution (Jacoby, 1978; Mannervik, 1985), associate as dimers with the subunits related by local twofold symmetry axes. In the crystal structures, regional conformational variations (Ji *et al.*, 1992) have been observed between the chemically identical subunits due in part to different crystal packing interactions.

During the crystallization trials of the μ-class enzyme, we observed two new crystal forms, each with a new and unique molecular packing environment. These new crystals offer an opportunity to compare structures of the same GST molecule crystallized with three different sets of crystal packing interactions. This information may help us to describe the structure of GST in solution which is free from crystal packing interactions, and to understand the significance, if any, of the subtle structural changes observed among different classes of GSTs. We report here the preliminary crystal data and the crystal packing analysis for these new crystal forms.

Experimental

A full-length μ-class GST 3 cDNA clone of rat liver was expressed in *Spodoptera frugiperda* (Sf 9) cells using a baculovirus expression system (Hsieh, Liu, Chen & Tam, 1989) which allowed isolation of large quantities of functionally active homogeneous GST 3-3 dimers of high purity without contamination by the closely related isoenzyme(s). The expressed protein was purified using the procedures of Mannervik & Guthenberg (1981).

For the crystallization set-ups, samples were dialyzed against 20 m*M* Tris–HCl buffer (pH 7.5) containing 20 m*M* NaCl, 1 m*M* EDTA and 0.02%(*w*/*v*) sodium azide, and then concentrated using Minicon cells (AMICON), to approximately 20–25 mg ml^{-1} as judged by UV absorbance. Crystallizations were carried out using the hanging-drop method (McPherson, 1982), with 20%(*w*/*v*) PEG 3350 used as the precipitant in 100 m*M* Hepes buffer (pH 8.0). The mother liquor also contained 10 m*M* β-octylglucopyranoside (β-OG) and 0.6 m*M* ethylmercury chloride which had proved essential for the growth of the form *A* crystals (Fu *et al.*, 1991). Set-ups were maintained at a temperature of 291 K. Using the above procedure, crystals suitable for X-ray diffraction analysis appear in one week (see Fig. 1).

The X-ray diffraction experiments were carried out with 5.4 kW Cu $K\alpha$ radiation generated using a Rigaku RU200 rotating anode. Data sets (Table 1) were collected on a Siemens X100 area detector utilizing the Harvard *COLLECT* routine (Blum, Metcalf, Harrison & Wiley, 1987). Crystal orientation, intensity integration and scaling were performed using *XENGEN* version 2.0 (Howard, Gilliland, Finzel, Poulos, Ohlendorf & Salemme, 1987).

MERLOT (Fitzgerald, 1988) was used to calculate the cross-rotation and translation functions. The search model used was the GST dimer extracted from the GST 3-3/GSH binary complex recently refined at 2.2 Å by Ji *et al.* (1992), Protein Data Bank entry 1GST. All side chains were included in the calculations. The model was initially rotated by $\alpha = 127.0$, $\beta = 52.0$, $\gamma = 194.0°$, so that the results

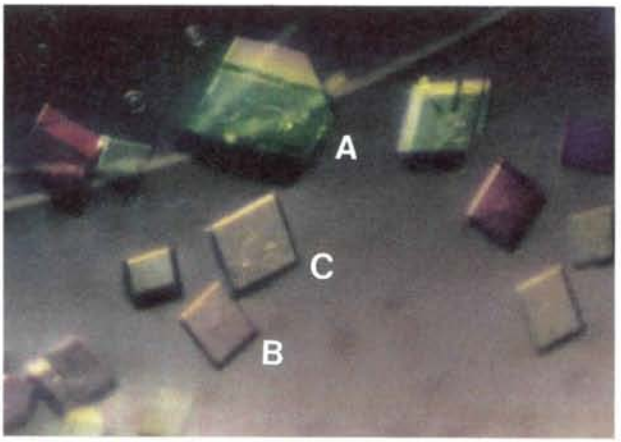

Fig. 1. Multiple crystal forms of rat liver GST 3-3, grown under the same conditions: 20% PEG 3350, 10 m*M* β-OG, 0.6 m*M* EtHgCl, 100 m*M* Hepes, pH 8.0.

would not fall into a poorly behaved region ($\beta \simeq 180°$) of rotation space. All $3\sigma_I$ data in the range 10–4 Å resolution were used in the calculation. The Patterson radius for the Crowther fast-rotation function was 23.9 Å. Once a solution had been found, a subsequent run of Lattman rotation function with a step size of 1° was performed over an area of angular dimensions of about $\Delta\alpha = \Delta\beta = \Delta\gamma = 14°$, centered on the solution, in order to gain better estimates of the Eularian angles. The results were then put into calculation of the $T(1)$ translation function (Crowther & Blow, 1967). The rotation and translation solutions were then subjected to a R-value minimization refinement (Ward, Wishner, Lattman & Love, 1975) for final adjustment.

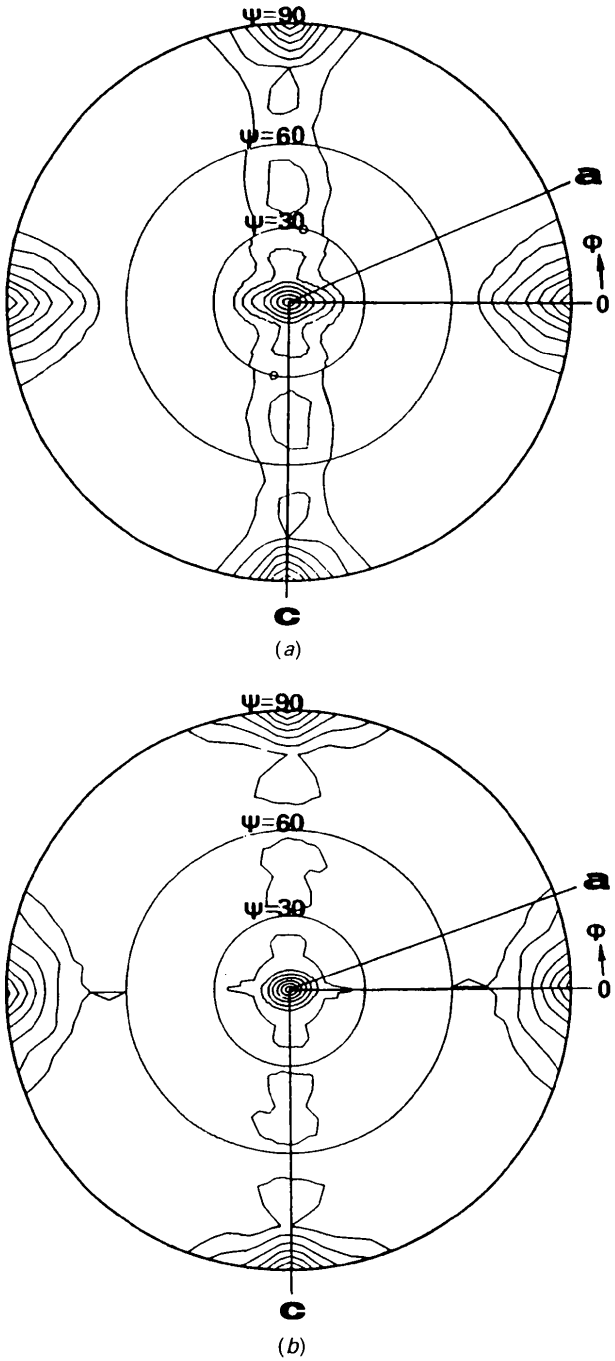

Fig. 2. The self-rotation functions, $\kappa = 180°$: (*a*) form *B* crystals of GST 3-3; (*b*) form *C* crystals of GST 3-3. The *b* axes are perpendicular to the paper. For the calculation, data to 5 Å were used with a Patterson radius of 30 Å.

Results and discussion

The new crystals were grown under essentially the same conditions as those used for the previously reported *C*2 crystal form, form *A* (Fu *et al.*, 1991). β-OG is essential for crystals of all the forms to grow large enough and to have good morphology.

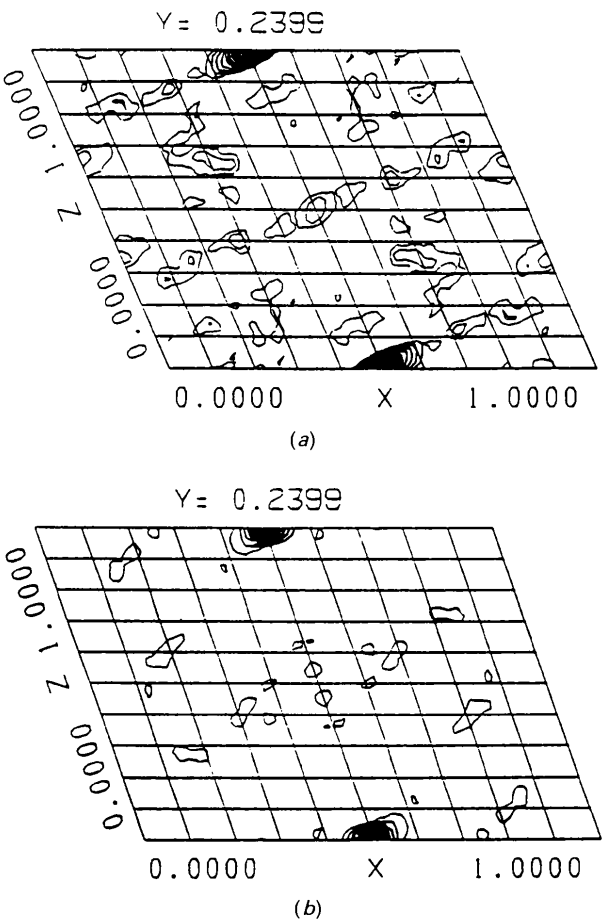

Fig. 3. Native Patterson maps: (*a*) form *B* crystal; (*b*) form *C* crystal at section $V = 0.24$. The large peaks were interpreted to represent the translation vectors between two dimers in the asymmetric unit. Note the similar location of the peaks in the two cases.

The inclusion of ethylmercury chloride is required for growing crystals of forms *A*, *B* and *C* with suitable unit-cell edges. Crystals obtained without this reagent, although in the presence of β-OG, had very large unit-cell constants (see Fu *et al.*, 1991) making it difficult for structure solving. So β-OG, necessary for large size and good quality of the crystals, was not responsible for promoting the growth in the three crystal forms discussed here. Both new crystal forms belong to space group $P2_1$ as judged by their systematic absences in $0k0$ for $k = 2n + 1$ and have two GST dimers per asymmetric unit. They are designated as form *B* and form *C*, respectively. The form *A* crystals have cell constants $a = 88.3$, $b = 69.7$, $c = 81.4$ Å and $\beta = 105.3°$; form *B* crystals have cell constants $a = 101.6$, $b = 69.5$, $c = 81.4$ Å, $\beta = 113.6°$; and form *C* crystals have cell constants $a = 97.4$, $b = 81.1$, $c = 69.4$ Å, $\beta = 109.2°$. Assuming one molecule per asymmetric unit, the calculated V_m is 2.3 Å^3 Da^{-1} and solvent content is 47% for form *A*. For both form *B* and form *C*, $V_m = 2.5$ Å^3 Da^{-1} and solvent content = 51% with the assumption that there are two molecules per asymmetric unit. It is interesting to note that all these crystal forms share similar cell dimensions (81 and 69 Å) along their unit-cell edges.

A Patterson self-rotation search for the form *B* crystals (Fig. 2*a*), yielded two peaks on the $\kappa = 180°$ section, at $\psi = 90°$, $\varphi = 0$ and 90° with heights equal to 92% of the crystallographic origin peak. Of these two peaks, one represents the 'true' direction of the non-crystallographic twofold axis, while the other is generated from the 'true' peak by interactions with the crystallographic twofold symmetry axis. This would suggest that the two dimers in the asymmetric unit have essentially the same orientation and they are related by a translation in the cell. This was affirmed by the observation of a large peak close to $U = 0.5$, $V = 0.24$, $W = 0.0$ in the native Patterson map (Fig. 3*a*).

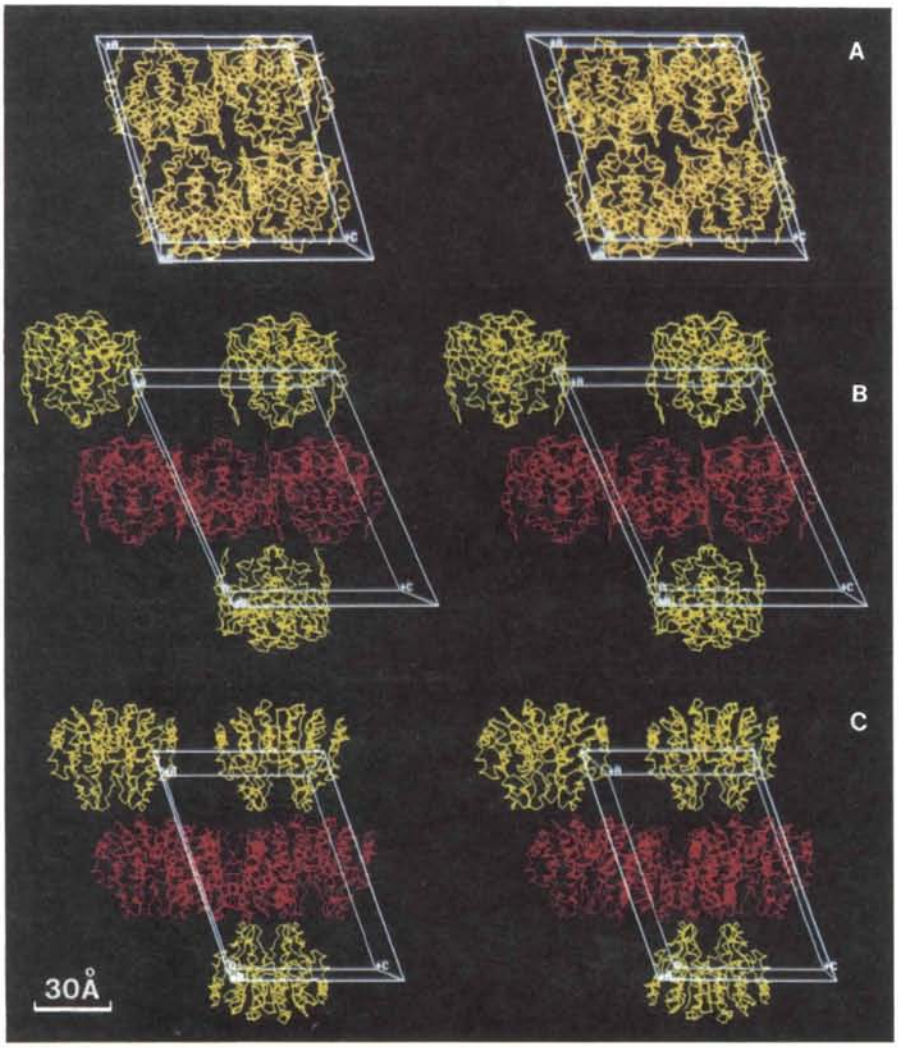

Fig. 4. Molecular packing diagrams (in stereo) of GST 3-3 dimers (C^α traces) in the unit-cells of: top, form *A* crystal containing one dimer (yellow) per asymmetric unit, showing the content of 1 unit cell; middle, form *B* crystal containing two (yellow and red) dimers per asymmetric unit, showing the content of $1\frac{1}{2}$ unit cells; and bottom, form *C* crystal containing two (yellow and red) dimers per asymmetric unit, showing the content of $1\frac{1}{2}$ unit cells.

The form *C* crystals showed a similar pattern in the self-rotation map (Fig. 2*b*) and native Patterson map (Fig. 3*b*) leading to the same general idea about the orientations of the two dimers in the asymmetric unit. However, since there is an interchange of cell lengths for the *b* and *c* axes between the two crystal forms, the dimers must be oriented differently in form *B* and form *C* unit cells. In fact, cross-rotation searches, as described below, show that the relative orientations of dimers in form *B* and form *C* crystals differ by nearly 90° about their molecular twofold axes.

For the form *B* crystals, the Crowther rotation function gave significant peaks at either $\alpha = 70.0$, $\beta = 98.0$, $\gamma = 135.0°$, height = 100%, or $\alpha = 110.0$, $\beta = 82.0$, $\gamma = 315.0°$, height = 100% with the next highest peak at 51%. These two solutions are essentially equivalent since they are related by the non-crystallographic twofold symmetry axis. The rotation axis calculated from the two peaks is a diad and coincides with the direction of the non-crystallographic twofold axis observed in the self-rotation studies. The first solution was then used to calculate the translation function and yielded a unique solution with $F_a = 0.483$, $F_b = 0.000$, $F_c = 0.243$ for dimer 1 and $F_a = 0.959$, $F_b = 0.224$, $F_c = 0.233$ for dimer 2. The position of the cross-vector peak agreed very well with the large peak observed in the native Patterson map. Values for the refined rotation and translation parameters are $\alpha = 68.48$, $\beta = 98.30$, $\gamma = 134.90°$, $F_a = 0.4810$, $F_b = 0.000$, $F_c = 0.2405$ for dimer 1, and $\alpha = 68.88$, $\beta = 101.50$, $\gamma = 139.50°$, $F_a = 0.9650$, $F_b = 0.2330$, $F_c = 0.2330$ for dimer 2, with $R = 40\%$ (60% initially).

For the form *C* crystals, the cross-rotation function again yielded two solutions at either $\alpha = 80.0$, $\beta = 68.0$, $\gamma = 50.0°$, height = 100%, or $\alpha = 100.0$, $\beta = 110.0$, $\gamma = 230.0°$, height = 100% with the next highest peak at 57%. For the reasons described above for the form *B* crystals, the two dimers also shared essentially the same rotation angles. Using the first solution, the translation function gave a unique result with $F_a = 0.483$, $F_b = 0.000$, $F_c = 0.243$ for dimer 1 and $F_a = 0.987$, $F_b = 0.243$, $F_c = 0.243$ for dimer 2. Again, position of the cross vector between the two dimers in an asymmetric unit obtained from the translation function agreed with the large peak in the native Patterson map. Refined values are $\alpha = 79.22$, $\beta = 68.50$, $\gamma = 51.50°$, $F_a = 0.4680$, $F_b = 0.000$, $F_c = 0.2362$ for dimer 1, and $\alpha = 81.22$, $\beta = 69.10$, $\gamma = 47.50°$, $F_a = 0.9850$, $F_b = 0.2400$, $F_c = 2430$ for dimer 2, with $R = 41\%$ (57% initially).

Based on the refined rotation parameters, the angular relationships between the two dimers in the asymmetric unit were calculated. For form *B* crystals, $\varphi = 90.20$, $\psi = 89.33$, $\kappa = 174.62°$, for form *C* crystals, $\varphi = 89.72$, $\psi = 89.39$, $\kappa = 183.57°$. Obviously, the directions of the axes between the dimers in the two crystal forms are basically the same, and that direction is, as dictated by their φ, ψ values, along their a^* axes. However, the relative rotations of the dimers about their axes in forms *B* and *C* differ by nearly 90° (Fig. 4). This relationship provided the basis for understanding the interesting observation that the cell lengths of the *b* and *c* axes were interchanged between the two crystal forms.

For the purpose of establishing the orientational relationship between the form *A* crystal and the form *B* and form *C* crystals, the procedures described above were also applied to form *A* crystal data, using the same starting model. The results indicate that the molecular orientations of the dimers are almost the same for the form *A* and form *B* crystals. What is different is the relative positioning of dimers along their local twofold axes. Interestingly, in form *B* and form *C* crystals the relative positioning of the dimers along the local twofolds are essentially the same, but their orientations are related to each other by nearly 90° as described above. The molecular packing diagrams of form *A*, *B* and *C* crystals are shown in Fig. 4. It will be of interest to compare the detailed structures of the dimers in the multiple crystal forms, as well as of the subunits of the two crystallographically independent dimers in the form *B* and form *C* crystals. A complete crystallographic refinement for these new crystal forms is in progress.

We thank Dr Xinhua Ji and Dr Gary Gilliland for kindly providing us with the coordinates of the refined GST 3-3/GSH complex structure. Unrefined C^α coordinates for these structures have been deposited with the Protein Data Bank.*

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1GSB, R1GSBSF and 1GSC, R1GSCSF). Free copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Supplementary Publication No. SUP 37100). At the request of the authors, the atomic coordinates will remain privileged until 1 March 1994 and the structure factors will remain privileged until 31 December 1995. A list of deposited data is given at the end of this issue.

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