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SHORT COMMUNICATIONS

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Crystals of isoenzyme 3-3 of rat liver glutathione S-transferase with and without inhibitor.

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Abstract

The isoenzyme 3-3 of rat liver glutathione S-transferase (GST 3-3) isolated from a baculovirus expression system has been crystallized with and without inhibitor. The crystals grown in the absence of an inhibitor belong to space group $P2_1$ with cell dimensions $a = 119{\cdot}7$, $b = 96{\cdot}2$, $c = 136{\cdot}7$ Å and $\beta = 103{\cdot}3°$, and diffract to 3 Å resolution. The crystals grown in the presence of an inhibitor belong to space group $C2$ with cell dimensions $a = 88{\cdot}3$, $b = 69{\cdot}7$, $c = 81{\cdot}4$ Å and $\beta = 105{\cdot}3°$, and diffract to at least 2·5 Å resolution. The inhibitor used is either methylmercury chloride or ethylmercury chloride; both are weak inhibitors.

Introduction

Glutathione S-transferases (GST's, E.C. 2.5.1.18) are a group of dimeric proteins, catalyzing the conjugation of glutathione to a wide variety of electrophilic alkylating agents. They are also involved in the reduction of organic hydroperoxides, isomerization of prostaglandins and binding of non-substrate hydrophobic ligands such as bile acids, bilirubin, a number of drugs and thyroid hormones. These isoenzymes are believed to be responsible for the detoxification of chemical carcinogens such as xenobiotics and endogenous compounds, as well as metabolic products from oxidative metabolism. A recent review on the structure and catalytic activity of GST has been given by Mannervik & Danielson (1988).

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The diversity of the enzyme's action provides a unique opportunity to study the relationship between structure and the mechanism of molecular detoxification. An understanding of the structure–function relationship will require the detailed structural information of GST's and their conformational changes. Crystallization and preliminary X-ray diffraction studies have been reported for several classes of GST complexed with inhibitors of glutathione (GSH) analogs (Sesay, Ammon & Armstrong, 1987; Schaffer, Gallay & Ladenstein, 1988; Cowan *et al.*, 1989; Parker, Bello & Federici, 1990). To our knowledge, there are no reports on crystals of GST uncomplexed with inhibitor. We report here the crystallization of rat liver GST 3-3 without inhibitor and in the presence of two non-GSH-based weak inhibitors.

Experimental

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

For the crystallization set-ups, the sample was dialyzed against 20 m*M* tris-HCl buffer (pH 7·0) containing 20 m*M* NaCl, 1 m*M* EDTA and 0·02% (*w*/*v*) sodium azide, and then concentrated to about 20 mg ml^{-1}. Crystallization

was carried out at 291 K in the presence of 20% (*w*/*v*) PEG 3350 by the hanging-drop method (McPherson, 1982). Crystals can be grown to sizes up to 0·1 × 0·3 × 0·7 mm in one week.

For the X-ray analysis, a crystal was mounted in a thin-walled glass capillary containing a small amount of mother liquor to prevent dehydration and sealed with wax. A data set to 3·2 Å resolution was collected on a Siemens X100 area detector using double mirror focused 5 kW Cu $K\alpha$ X-rays generated from a Rigaku RU200 rotating anode. The data collection was carried out using the Harvard *COLLECT* routines (Blum, Metcalf, Harrison & Wiley, 1987). Crystal orientation, integration and scaling were performed using the *XENGEN* (Howard, Gilliland, Finzel, Poulos, Ohlendorf & Salemme, 1987) program suite.

Results and discussion

Analysis of the three-dimensional data set indicates a monoclinic space group with cell parameters $a = 119{\cdot}7$, $b = 96{\cdot}2$, $c = 136{\cdot}7$ Å and $\beta = 103{\cdot}3°$. Systematic absences in $0k0$ for $k = 2n + 1$ suggest that the space group is $P2_1$. The unit-cell volume (1 558 237 Å^3) implies that there may be five or six dimers of 52 000 daltons each per asymmetric unit with volume per mass ratio, V_m (Matthews, 1968) about 3·0 and 2·5 respectively. Both are within the normal range for protein crystals. These crystals represent the first crystallization of GST in the absence of inhibitor.

When the mother liquor contained methylmercury chloride or ethylmercury chloride with the presence of β-octylglucopyranoside (10 m*M*), crystals of a different morphology were obtained. These crystals diffract to at least 2·5 Å resolution. Methylmercury chloride (MeHgCl) has been reported as an inhibitor to GST (Reddy, Scholz & Massaro, 1981). Following the activity assay method of Habig & Jakoby (1981) we found that complete inhibition is observed at 1 m*M* MeHgCl and 90% inhibition is observed at 0·6 m*M* MeHgCl. Ethylmercury chloride (EtHgCl), however, is less effective as an inhibitor, giving only 24·4 and 14·6% inhibition at 0·6 and 0·1 m*M* EtHgCl respectively. Diffraction data sets were collected on a Siemens area-detector system for crystals co-crystallized with both the MeHgCl and EtHgCl using procedures similar to those mentioned above for the $P2_1$ crystals. Analysis of the three-dimensional data indicates that crystals of the MeHgCl and EtHgCl complexes are isomorphous and belong to space group $C2$ with cell parameters, $a = 88{\cdot}3$, $b = 69{\cdot}7$, $c = 81{\cdot}4$ Å and $\beta = 105{\cdot}3°$. Assuming one dimer per asymmetric unit, the V_m is calculated to be 2·32 and the solvent content is estimated to be 47%.

These $C2$ crystals represent the weakly or partially inhibited GST's. They appear to be isomorphous with those of rat liver GST 3-3 grown (Sesay *et al.*, 1987) in the presence of a glutathione-based inhibitor. It would be interesting to compare these structures as well as that of the $P2_1$ crystals which contain no inhibitors. Crystallographic studies of both crystal forms of GST are underway.

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Crystallographic refinement of bovine pro-phospholipase A_2 at 1·6 Å resolution.

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Abstract

Bovine pro-phospholipase A_2 ($M_r = 14\,520$), trigonal, $P3_121$, $a = b = 46{\cdot}5$, $c = 102{\cdot}0$ Å, one molecule per asymmetric unit, λ(Cu $K\alpha$) = 1·54 Å. The model incorporating 895 protein atoms, two molecules of 2-methyl-2,4-pentanediol, and 60 solvent water molecules, was refined by restrained least squares to a residual $R = 0{\cdot}194$ for 14 667 reflections from 5 to 1·6 Å resolution.

Introduction

Phospholipase A_2 (PLA2; EC 3.1.1.4) is a small enzyme catalyzing fatty acid hydrolysis from the *sn*-2-position of

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