γ-Glutamylamine cyclotransferase (GGACT) is an enzyme that converts γ-glutamylamines to free amines and 5-oxoproline. GGACT shows high activity toward γ-glutamyl-ε-lysine, derived from the breakdown of fibrin and other proteins cross-linked by transglutaminases. The enzyme adopts the newly identified cyclotransferase fold, observed in γ-glutamylcyclotransferase (GGCT), an enzyme with activity toward γ-glutamyl-α-amino acids (Oakley, A. J., Yamada, T., Liu, D., Coggan, M., Clark, A. G., and Board, P. G. (2008) J. Biol. Chem. 283, 22031–22042). Despite the absence of significant sequence identity, several residues are conserved in the active sites of GGCT and GGACT, including a putative catalytic acid/base residue (GGACT Glu82). The structure of GGACT in complex with the reaction product 5-oxoproline provides evidence for a common catalytic mechanism in both enzymes. The proposed mechanism, combined with the three-dimensional structures, also explains the different substrate specificities of these enzymes. Despite significant sequence divergence, there are at least three subfamilies in prokaryotes and eukaryotes that have conserved the GGCT fold and GGCT enzymatic activity.

Proteins can be cross-linked via the side chains of glutamine and lysine by transglutaminases. This reaction results in the formation of ammonia and an L-γ-glutamyl-L-ε-lysine isopeptide bond linking the two polypeptide chains. The existence of such γ-glutamyl-ε-lysine links was unambiguously demonstrated in factor XIIIa-cross-linked fibrin: extensive proteolytic degradation of cross-linked fibrin resulted in the formation of L-γ-glutamyl-L-ε-lysine (1). It was concluded that L-γ-glutamyl-L-ε-lysine is not broken down by conventional proteolysis. The breakdown of the isopeptide is instead catalyzed by γ-glutamylamine cyclotransferase (GGACT),\(^2\) first purified from rabbit kidney (2, 3). The partially purified enzyme was demonstrated to be active toward a range of L-γ-glutamyl conjugates with mono- and polyamines and amino acids. The action of GGACT in all cases is to cyclize the γ-glutamyl moiety, producing 5-oxo-L-proline and the free alkylamine. No activity was detected toward L-glutamine or L-β-aspartyl-L-ε-lysine. Furthermore, derivatives of L-γ-glutamyl-L-ε-lysine in which the α-amino or α-carboxyl functional group of the glutamyl moiety is blocked do not serve as substrates, nor do any of a range of L-γ-glutamyl-1-α-amino acids (3). Based on these results, it was proposed that GGACT functions in the latter stages of the catabolism of the products of transglutaminases (3).

We reported recently the identification, cloning, and three-dimensional structure of an enzyme with related catalytic activity to GGACT but with distinct specificity: γ-glutamylcyclotransferase (GGCT) (4). Unlike GGACT, this enzyme is active toward a range of L-γ-glutamyl-α-amino acids (5, 6). GGCT catalyzes the penultimate step in glutathione catabolism, whereby L-γ-glutamyl-1-α-cysteine and other L-γ-glutamyl-L-α-amino acid dipeptides formed by the γ-glutamyl cycle are catabolized to 5-oxo-L-proline and a free amino acid. (L-Glutamatic acid is subsequently formed from 5-oxo-L-proline by the action of 5-oxoprolinase.) We demonstrated that GGCT is a homodimer of 20,994-Da subunits and has a distinctive mixed α/β-topology with six β-strands, five α-helices, and four short 3_10 helices, with strands β1–5 forming a barrel structure (4). This fold has also been observed in several proteins of unknown function from animals, plants, bacteria, and archaea. One such structure, solved by the Joint Center for Structural Genomics Consortium, is a mouse protein (AIG2-like domain 1; Protein Data Bank code 1VKB) of unknown function (7). The gene encoding this protein is distinct from the mouse gene encoding GGCT. Furthermore, a homolog of mouse AIG2-like domain 1 known as A2LD1 was predicted to exist in the human genome. The structure of the mouse protein possesses a cleft similar to the proposed active site of human GGCT. Tantalizingly, the proposed catalytic residue (Glu98 in human GGCT) and other active-site residues are structurally conserved in the mouse protein and in the amino acid sequence of its human homolog. These observations led us to the hypothesis that this mouse protein and its human homolog are also cyclotransferases, possibly the long sought after GGACT. Here, we describe the cloning, expression, purification, catalytic activity, and three-dimensional structure of human A2LD1 and positively identify it as GGACT. A common mechanism is proposed to act in both
GGCT and GGACT. The proposed mechanism, combined with the three-dimensional structural data, also explains the different substrate specificities of these enzymes.

**EXPERIMENTAL PROCEDURES**

**Cloning—**A human expressed sequence tag clone (GenBank accession number BU156875) from a melanoma cell line was identified by sequence alignment with a mouse protein with a GGCT-like fold (Protein Data Bank code 1VKB). The clone was obtained from the I.M.A.G.E. Consortium, and the coding region of the cDNA was amplified with primers GGACT-F (5’-CTCCGGCGTGGAATGGCCCTAGTCTTCGTG-3’) and GGACT-R (5’-CTAAGCTTATCATCTGGTTCTGCCGGG-3’) and cloned into pGEM-T vector with KspI and then ligated into the same sites in the pHUE cDNA was excised from the pGEM-T vector with KspI and HindIII and then ligated into the same sites in the pHUE cloning Biomedical Resource Facility of the John Curtin School clone was sequenced by the Australian Cancer Research Foundation.

**Expression**—The human GGACT gene (GenBank accession number BU156875) from a melanoma cell line was obtained from the I.M.A.G.E. Consortium, and the coding sequence alignment, which was edited for clarity.

**Gene, Protein, and Enzymatic Characterization—**Examination of NCBI Human Genome Database Entrez Gene entries showed that the GGACT gene (A2LD1, Gene ID877769) is quite small (2,187 kb) and is located on chromosome 13q32.3. It is interesting to note that the gene contains only a single intron that falls within the 5’-noncoding region of the gene transcript. The human GGACT gene (A2LD1) encodes a protein of 153 amino acids with a predicted molecular mass of 17,327 Da. The human A2LD1 CDNA was expressed in E. coli as a 17,300-Da protein. SDS-PAGE of the purified recombinant enzyme under reducing conditions showed a protein that migrated within the expected size range. Gel filtration of the native protein revealed a molecular mass of 18 kDa, which is compatible with a monomeric structure (data not shown). Analysis of the potential γ-glutamylcyclotransferase activity of the recombinant enzyme revealed that it is inactive with i-γ-glutamyl-α-amino acid substrates such as i-γ-glutamyl-1-α-cysteine and i-γ-glutamyl-l-α-alanine but has distinct activity with i-γ-glutamyl-1-l-lysine (2.7 ± 0.06 μmol/min/mg). In contrast, recombinant human GGCT is active with i-γ-glutamyl-1-α-alanine (50.3 ± 1.22 μmol/min/mg) but inactive with i-γ-glutamyl-l-lysine. As a result of this clear specificity, we will refer to the product of the so-called A2LD1 gene as γ-glutamylaminocyclotransferase.

**Crystal Structure—**Crystals of human GGACT appeared in several polyethylene glycol-based conditions in the JCSG and PACT screens. The best crystals grew at 8 °C with JCSG condi-
Structure of \( \gamma \)-Glutamylamine Cyclotransferase

Table 1

Crystallographic data

| PDB code | Structure |
|----------|-----------|
| 3JUB | GGACT | 3JUC | GGACT | 5-oxoproline | 3JUD | GGACT E82Q mutant |
| X-ray data | | | | | |
| Beamline | PX-1 | PX-1 | PX-2 | | |
| Space group | P2\(_1\), 2\(_1\), 2\(_1\) | P2\(_1\), 2\(_1\), 2\(_1\) | P2\(_1\), 2\(_1\), 2\(_1\) | | |
| Unit cell parameters | \( a = 36.0, b = 41.9, c = 84.0 \) \( \text{Å} \); \( \alpha = \beta = \gamma = 90° \) | \( a = 36.2, b = 42.5, c = 84.8 \) \( \text{Å} \); \( \alpha = \beta = \gamma = 90° \) | \( a = 36.3, b = 42.4, c = 84.8 \) \( \text{Å} \); \( \alpha = \beta = \gamma = 90° \) | | |
| Resolution range (Å) | 30–1.2 (1.26–1.20) | 43–1.2 (1.26–1.20) | 38–0.98 (1.03–0.98) | | |
| Total no. of observations | 277,499 (38,387) | 369,388 (39,839) | 494,078 (72,260) | | |
| No. of unique reflections | 40,593 (5782) | 40,928 (5805) | 74,200 (10,958) | | |
| Bond lengths (Å) | 0.023 | 0.022 | 0.029 | | |
| General planes (Å) | 0.012 | 0.011 | 0.013 | | |
| \( R \)merge (%) | 22.2 (5.2) | 27.5 (10.7) | 10.4 (2.5) | | |
| \( R \)merge (%) | 5.8 (32.3) | 5.5 (15.4) | 10.4 (68.8) | | |
| Completeness | 99.9 (97.0) | 98.5 (97.0) | 97.7 (100.0) | | |
| Multiplicity | 6.8 (6.6) | 9.0 (6.9) | 6.7 (6.8) | | |
| \( B \) from Wilson plot (Å\(^2\)) | 8.7 | 8.7 | 5.0 | | |

Refinement statistics

| PDB code | Structure |
|----------|-----------|
| 3JUB | GGACT | 3JUC | GGACT | 5-oxoproline | 3JUD | GGACT E82Q mutant |
| X-ray data | | | | | |
| Resolution range (Å) | 42–1.20 (1.23–1.20) | 42–1.20 (1.23–1.20) | 42–0.98 (1.01–0.98) | | |
| No. of reflections used in refinement | 38,497 (2917) | 38,832 (2906) | 70,357 (5455) | | |
| No. of reflections (\( R_{ave} \) set) | 2032 | 2053 | 3736 | | |
| \( R \)work (%) | 12.3 (14.9) | 11.7 (14.8) | 15.3 (23.9) | | |
| \( R \)free (%) | 13.7 (17.0) | 9.5 (12.8) | 18.0 (26.0) | | |
| No. of atoms | 1433 | 1514 | 1450 | | |
| \( B \) of structure (Å\(^2\)) | 9.0 | 9.8 | 8.4 | | |
| r.m.s.d. from ideal geometry | | | | | |
| Bond lengths (Å) | 0.023 | 0.022 | 0.029 | | |
| Bond angles | 1.91° | 1.92° | 2.31° | | |
| Chiral centers (Å\(^2\)) | 0.132 | 0.111 | 0.153 | | |
| General planes (Å) | 0.012 | 0.011 | 0.013 | | |

Numbers in parentheses refer to the highest resolution bin.

Angle brackets refer to mean values.

\( R \)merge = \( \sum_{i} \sum_{j} |F_{o,i} - F_{c,i}| / \sum_{i} \sum_{j} |F_{o,i}| \), where \( F_{o,i} \) and \( F_{c,i} \) are the observed and calculated structure factors, respectively. \( R_{ave} \) was calculated from 5% of the diffraction data not used in refinement.

The cavity is formed by Tyr\(^7\), Gly\(^8\), and Thr\(^9\) in the loop following strand B\(^1\) (a conserved motif in the cyclotransferase family; see below), Leu\(^10\), Glu\(^82\), Tyr\(^88\), and Tyr\(^119\). The secondary amine group of oxoproline is 3.2 Å from the Glu\(^82\) carboxylate group. The loop containing residues 7–10 has a backbone conformation that causes the main chain amino groups to be oriented into the cavity. The oxoproline carboxylic acid moiety accepts hydrogen bonds from the main chain amino groups of Tyr\(^7\) and Gly\(^8\). The carbonyl oxygen of oxoproline accepts a hydrogen bond from the backbone amino and side chain O–γ moieties of Thr\(^9\). The aromatic face of the side chain of Tyr\(^7\) forms the bottom of the cavity. The ligand 5-oxo-L-proline sits \(~\)3.6 Å from this residue (Fig. 3). Using this structure as a template, the substrate \( \gamma \)-glutamyl-L-ε-lysine has been modeled into the active site (Fig. 3B). Similarly, substrate or oxoproline has been modeled in the active site of GGCT (Fig. 3, C and D). Patterns of hydrogen bonding are conserved in these structures.

Site-directed Mutagenesis—We demonstrated previously that Glu\(^82\) is crucial for catalytic activity in GGCT (4). To determine the importance of the equivalent residue in GGACT (Glu\(^82\)), we mutated this residue to glutamine or alanine. The E82Q and E82A mutants had no catalytic activity, and the E82A enzyme was unstable and readily precipitated. We crystallized the E82Q mutant under conditions identical to those used for the wild-type enzyme (Fig. 1C). The E82Q mutant and wild-type structures superimpose with a root mean square deviation of 0.352 Å over 144 C-α atoms. The only difference in the active site is a small rotation of Glu\(^82\) about the C-γ–C-δ bond with respect to Glu\(^82\). The binding patterns of the water molecule and an NO\(_3\) group binding in the active site are identical in the mutant and wild-type structures.
Comparison of GGACT with Related Structures—A DALI search for structural homologs revealed a limited number of proteins with this fold. These were from both prokaryotic and eukaryotic species and included *Pyrococcus horikoshii* (Protein Data Bank code 1V30), *E. coli* (code 1XHS), *Arabidopsis thaliana* (code 2G0Q), and *Bacillus subtilis* (code 2QIK), as well as the previously identified human GGCT and mouse GGACT homologs (codes 2PN7 and 1VKB, respectively). The superposed structures and structure-based sequence alignment (Fig. 4) show several conserved features: a β-barrel topology with two strands “crossing over” as described above, a highly conserved helix (in GGACT numbering, helix H9251), a binding cavity formed on one side of the β-barrel, a loop following strand H9252 containing a conserved (V/A)YG(S/T) motif, a conserved tyrosine in strand H9254, and an aromatic residue in strand H9255.

Phylogenetic Relationships—Despite the conservation of the overall fold and the active-site architecture, there is considerable sequence divergence between the different proteins exhibiting the GGCT fold. A direct alignment of the sequence of human GGACT with that of human GGCT showed <10% amino acid identity (Fig. 4). Similarly, pairwise alignment of the GGCT and GGACT sequences with the prokaryotic members of this structural family revealed a similar low level of sequence identity. To gain some insight into the evolution of this fold and the diversity within this broad protein family, we undertook BLAST searches to identify homologous proteins in different species. Multiple sequence alignments using the COBALT alignment tool (NCBI) (19) were then used to build a phylogenetic tree. Although there are many sequences available for this

![Figure 1](image1)
**FIGURE 1.** Models of GGACT in the vicinity of the active site shown in stick form with 2mFo – DFc electron density, contoured at 1σ shown in chicken wire representation. A and B, GGACT and GGACT, respectively, in complex with 5-oxo-L-proline; C, GGACT E82Q mutant.

![Figure 2](image2)
**FIGURE 2.** A, schematic representation of GGACT, with bound nitrate groups represented in stick form. The N and C termini are labeled. B, topology diagram of GGACT. Interactions between β-strands are indicated by black rectangles. The broken rectangles adjacent to strands β2a and β4 indicate the wrapping of the β-sheet to form a barrel.

![Figure 3](image3)
**FIGURE 3.** A, structure of the active site of GGACT in complex with 5-oxo-L-proline; B, model of GGACT in complex with L-γ-glutamyl-L-lysine; C, model of GGCT in complex with 5-oxo-L-proline; D, model of GGCT in complex with L-γ-glutamyl-L-α-cysteine. In all cases, carbon atoms of the protein and ligand are yellow and green, respectively. Potential hydrogen bonds are shown as thin black lines.
Structure of γ-Glutamylamine Cyclotransferase

**FIGURE 4. Sequence alignment of Protein Data Bank structures adopting the cyclotransferase fold.**
Sequences are labeled by Protein Data Bank codes, except for human GGACT (hGGACT) and GGCT (hGGCT). Protein Data Bank code 2QIK contains two cyclotransferase domains that have been split into 2QIK-1 and 2QIK-2. Secondary structure elements of human GGACT are denoted above the sequences. Where significant structural homology exists, the residues are shown in uppercase letters. Residues lining the active-site cavity are indicated with green triangles. Possible catalytic glutamate residues are highlighted with a red star. Completely conserved (black) and highly conserved (gray) residues are highlighted.

type of analysis, the sequences used here were selected to provide a broad evolutionary perspective. A neighbor joining tree (Fig. 5) indicated that there are three main families of proteins with the γ-glutamylcyclotransferase fold (GGCT-like, GGACT-like, and BtrG-like), and there is evidence that each group has retained γ-glutamylcyclotransferase activity. It is evident from the lack of sequence identity that these families have evolved separately for a considerable period. The GGCT-like family appears to be restricted to animals with sequences identified in a range of species from *Caenorhabditis elegans* to humans. Our previous studies showed that the human GGCT enzyme (Protein Data Bank code 2PN7) has γ-glutamylcyclotransferase activity (4). The GGACT-like family is also restricted to eukaryotes but clearly includes plants, as confirmed by the structure from *A. thaliana* (Protein Data Bank code 2GOQ). In this study, we have shown that members of the GGACT-like family have γ-glutamylcyclotransferase activity. In contrast, the BtrG-like family appears to be restricted to prokaryotes. The BtrG protein from *Bacillus circulans* has been shown to have γ-glutamylcyclotransferase activity in an antibiotic synthesis pathway (20), and the structures of the sequence-related proteins from *P. horikoshii* (Protein Data Bank code 1V30) and *E. coli* (code 1XHS) confirm the association between this fold and γ-glutamylcyclotransferase enzymatic activity in prokaryotes. Although we did not find evidence for GGCT fold proteins in yeast or fungal species, it is possible that they have diverged beyond the level of sequence identity that can be identified by sequence similarity searches, and these await identification via structural studies.

**DISCUSSION**

The enzymatic activity known as γ-glutamylamine cyclotransferase has been unambiguously assigned to the human gene known as *A2L.D1*. The human GGACT protein is clearly similar to the mouse protein AIG2-like domain 1, and the human and mouse genes are on syntenic chromosomes. The two proteins can be superimposed with a root mean square deviation of 0.68 Å over 138 C-α atoms, and the putative active-site residues are conserved. It is therefore likely that the mouse protein has GGACT activity. The structure of GGCT bound to the reaction product 5-oxo-1-proline (Figs. 1B and 3A) unambiguously identifies the active site. The GGACT residues in proximity to this entity, Tyr, Gly, Thr, Glu, and Tyr, are structurally equivalent to Tyr, Gly, Ser, Glu and Tyr in GGCT, respectively, strongly suggesting that this compound binds in an analogous fashion in both enzymes. A model of the 5-oxo-1-proline-GGCT complex was therefore produced (Fig. 3C). We propose that these residues form the binding site for the L-γ-glutamyl portion of the substrates of both enzymes (Fig. 3, B and D). In both models, the α- and γ-amino groups of the glutaminyl moieties of the substrates are in close proximity to Glu and Glu (GGACT) and Glu (GGCT). We propose that Glu (GGACT) and Glu (GGCT) share the same function, i.e. a general acid/base, and propose the following reaction mechanism: the α-amino group of the L-γ-glutamyl moiety is deprotonated by Glu/Glu, with the concomitant nucleophilic attack of this amine onto the side chain amide carbon atom. The resulting oxianion intermediate collapses to form 5-oxo-1-proline, with the now protonated Glu/Glu residue donating a hydrogen ion to the amine of the α- or ε-linked amino portion of the substrate (Fig. 6). We have demonstrated by site-directed mutagenesis and kinetics experiments that Glu/Glu (GGACT) and Glu/Glu (GGCT) (4) are essential for activity. The structural integrity of E82Q indicates
that a glutamyl residue at this position is not essential for folding. Our model of substrate binding in GGACT and GGCT can further explain the substrate specificities of both enzymes. In our models, the groups to which the glutamyl moieties are linked are orientated out of the active site and toward the surface, and the residues of GGACT and GGCT share little structural similarity in this vicinity. In our GGCT model with substrate, Arg30 is involved in a salt bridge with the \(-\)carboxylic acid of the cysteine residue, compatible with the known preference of GGCT for \(L\)-glutamyl-\(L\)-amino acid substrates. GGACT lacks an arginine residue at the equivalent location and does not act on \(L\)-glutamyl-\(L\)-amino acids. The active site is narrower in GGACT compared with GGCT, partly due to the presence of Phe\(^{81}\) (Fig. 7). This is compatible with the preference of GGACT for substrates with extended aliphatic amines (lysine, spermidine, etc.) conjugated to the \(\gamma\)-glutamyl group.

The \(B.\ subtilis\) homolog (named YqkA, of unknown function; Protein Data Bank code 2QIK) has two cyclotransferase domains contained within one polypeptide chain. Only the second has a glutamyl residue equivalent to Glu\(^{82}\) in GGACT. This protein possibly has cyclotransferase-like activity in its C-terminal domain, with a regulatory function in the N-terminal domain. The \(E.\ coli\) homolog (named YtfP, of unknown function; Protein Data Bank code 1XHS) has an arginine residue in place of GGACT residue Glu\(^{82}\), suggesting that this protein is not a cyclotransferase but might bind a similar substrate. The \(A.\ thaliana\) protein (known as At5g39720.1, of unknown function; Protein Data Bank code 2G0Q) is clearly a member of the GGCT structural family and has a conserved active-site glutamyl residue, suggesting that it may have cyclotransferase activity. This is consistent with the previous detection of \(\gamma\)-glutamylcyclotransferase activity in tobacco suspension cultures (21).

At this juncture, the lack of structural similarity of the GGCT family of cyclotransferases and the glutaminyl cyclases (QCs) (EC 2.3.2.5) should be noted. The QCs catalyze the formation of N-terminal 5-oxoproline from its glutaminyl (or glutamyl) precursor on peptides. This modification is required by certain proteins for biological function in either mediating interaction with receptors or stabilizing proteins against N-terminal degradations. QCs are classified as belonging to two groups: mammalian QCs and a second group containing enzymes from bacteria, plants, and parasites. The structure of human QC, representative of the first group, adopts an \(\alpha/\beta\)-open sandwich fold similar to the two-zinc exopeptidases (22) and requires zinc as a cofactor. Although human QC is unrelated to the GGCT family in either sequence or three-dimensional structure, it does appear to contain a catalytic acid/base residue (Glu\(^{201}\)) analogous in function to Glu\(^{82}\) in GGACT. In human QC, a Zn\(^{2+}\) ion is proposed to stabilize an oxyanion intermediate during catalysis. In GGACT, the Thr\(^{9}\) amino and side chain hydroxyl groups appear to perform this function. As with GGCT and GGACT, mutation of the proposed acid/base residue in human QC...
Structure of γ-Glutamylamine Cyclotransferase

(2E01Q) inactivates the enzyme. Papaya QC, representative of the second group, adopts a five-blade β-propeller (23). Although this enzyme binds a Zn$^{2+}$ ion, this does not appear to be involved in catalysis. Nevertheless, the proposed mechanism for papaya QC involves the use of an acid/base residue (Glu98) in an analogous fashion to Glu82 in GGACT, Glu19 in GGCT, and Glu201 in human QC. In the proposed mechanism, papaya QC stabilizes the oxyanion using the side chain amino group of Lys225. Taken together, the structural and functional data for QC, GGACT, and GGCT enzymes suggest convergent evolution in the catalytic mechanism.

The wide distribution of proteins adopting the cyclotransferase fold across Eukarya, Archaea, and Bacteria may indicate an ancient evolutionary origin of the cyclotransferase fold. The structural features most strongly conserved are the β-barrel and helix α1 (GGACT numbering). The STAMP alignment also shows key conserved sequence motifs. Despite the lack of significant sequence identity between GGCT and GGACT, the conservation of topology (including the unusual β-strand crossover described above) and active-site residues leads us to propose that these enzymes are derived from a common ancestral gene and functionally specialized after a gene duplication event, with GGACT optimized for catalysis on substrates with a γ-γ-glutamyl moiety linked to extended alkylamines and GGCT optimized for catalysis on substrates with a 1-γ-glutamyl moiety linked to 1-α-amino acids. There is recent evidence that some prokaryotic members of this structural family also have γ-glutamylcyclotransferase activity, as the BtrG protein from B. circulans catalyzes the formation of 5-oxoproline from an intermediate in the synthesis of the antibiotic butirosin (20). Thus, although the primary amino acid sequences of this structural family are incredibly diverse, the conserved fold is very strongly associated with conserved γ-glutamylcyclotransferase activity. Because there is such great sequence diversity between the three groups within this structural family, there is a strong possibility that additional subfamilies with sequence diversity beyond the reach of BLAST alignments may be identified by structural studies in the future.

Acknowledgments—We thank the technical staff of the Bio21-C3 Center for help with crystallization. This work was undertaken on the PX-1 and PX-2 beamlines at the Australian Synchrotron (Victoria, Australia).

REFERENCES

1. Pisano, J. J., Finlayson, I. S., and Peyton, M. P. (1968) Science 160, 892–893
2. Fink, M. L., Chung, S. L., and Folk, J. E. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 4564–4568
3. Fink, M. L., and Folk, J. E. (1981) Mol. Cell. Biochem. 38, 59–67
4. Oakley, A. J., Yamada, T., Liu, D., Coggan, M.,Clark, A. G., and Board, P. G. (2008) J. Biol. Chem. 283, 22031–22042
5. Orlowski, M., Richman, P. G., and Meister, A. (1969) Biochemistry 8, 1048–1055
6. Orlowski, M., and Meister, A. (1973) J. Biol. Chem. 248, 2836–2844
7. Klock, H. E., Schwarzenbacher, R., Xu, Q., Mcmullan, D., Abudubek, P., Ambing, E., Axelrod, B., Biorac, T., Canaves, J. M., Chiu, H. J., Deacon, A. M., DiDonato, M., Elslicher, M. A., Godzik, A., Grittini, C., Grzechniak, S. K., Hale, J., Hampton, E., Han, G. W., Haugen, J., Hornsby, M., Jarolaza, E., Koesema, E., Kreusch, A., Kuhn, P., Miller, M. D., Moy, K., Nigohgissian, E., Paulsen, J., Quinto, K., Reyes, R., Rife, C., Sims, E., Spraggon, G., Stevens, R. C., van den Bedem, H., Velasquez, J., Vincent, J., White, A., Wolf, G., Hodgson, K. O., Wooley, J., Lesley, S. A., and Wilson, I. A. (2005) Proteins 61, 1132–1136
8. Catanzariti, A. M., Soboleva, T. A., Tans, D. A., Board, P. G., and Baker, R. T. (2004) Protein Sci. 13, 1331–1339
9. Board, P. G., Moore, K. A., and Smith, J. E. (1978) Biochim. J. 173, 427–431
10. Board, P. G., Smith, J. E., and Moore, K. (1978) J. Lab. Clin. Med. 91, 127–131
11. Phillips, T. M., McPhillips, S. E., Chiu, H. J., Cohen, A. E., Deacon, A. M., Ellis, P. J., Garman, E., Gonzalez, A., Sauter, N. K., Phizackerley, R. P., Soltes, S. M., and Kuhn, P. (2002) J. Synchrotron Radiat. 9, 401–406
12. Leslie, A. G. (2006) Acta Crystallogr. D Biol. Crystallogr. 62, 48–57
13. Evans, P. (2006) Acta Crystallogr. D Biol. Crystallogr. 62, 72–82
14. Lebedev, A. A., Vagin, A. A., and Murshudov, G. N. (2008) Acta Crystallogr. D Biol. Crystallogr. 64, 33–39
15. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Acta Crystallogr. D Biol. Crystallogr. 53, 240–255
16. Emsley, P., and Cowtan, K. (2004) Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132
17. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 283–291
18. Russell, R. B., and Barton, G. J. (1992) Proteins 14, 309–325
19. Papadopouloos, I. S., and Agarwala, R. (2007) Bioinformatics 23, 1073–1079
20. Llewellyn, N. M., Li, Y., and Spencer, J. B. (2007) Chem. Biol. 14, 379–386
21. Stein Kamp, R., Schweinhofen, B., and Rennenberg, H. (1987) Physiol. Plant. 69, 499–503
22. Huang, K. F., Liu, Y. L., Cheng, W. J., Ko, T. P., and Wang, A. H. J. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 13117–13122
23. Wintjens, R., Belahi, H., Clantin, B., Azarkan, M., Bompard, C., Baeyens-Volant, D., Looose, Y., and Villeret, V. (2006) J. Mol. Biol. 357, 457–470