Structure of ã-glutamylcysteine Synthetase Complexed with Buthionine Sulfoximine

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ã-Glutamylcysteine synthetase (GCS) catalyzes the first and rate-limiting step of biosynthesis of a ubiquitous tripeptide glutathione and is a target for development of potential therapeutic agents against parasites and cancer. L-Buthionine-(*SR*)-sulfoximine (BSO) is a well-known potent inhibitor of GCS. Clinical trials of BSO have been carried out against alkylating or platinating agent resistance cancers. Crystallographic analyses of GCS-BSO complex will provide an important clue to the catalytic mechanism and structure-assisted drug design for any species of GCSs.

The crystal of *E. coli* GCS in complex with BSO belongs to the space group $P2_1$ with unit cell constants of a=70.5 Å, b=97.6 Å, c=102.7 Å and b=109.5°. The current model was refined to an R-factor of 21% (R_{free}=24%). g-Phosphate of ATP has already been transferred to the NS sulfoximine nitrogen atom of BSO. We have shown that the cysteine-binding site of the GCS is inductively formed at the binding of cysteine substrate with turn of side chains of Tyr-241 and Tyr-300 to make hydrogen bonds with the carboxyl group of cysteine that w-carboxyl group of BSO mimics. The binding of BSO to the enzyme induces the turn of the side chain of Tyr-241 in spite of the lack of BSO's w-carboxyl group. This conformational change of the side chain may be stabilized by van der Waals interaction between the side chain of Tyr-241 and the glutamate moiety in BSO.

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