A New Enzyme [EC 2.5.1.65], O-Phospho-L-serine Sulfhydrylase, from a Hyperthermophilic Archaeon *Aeropyrum pernix*

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A putative O-acetylserine sulfhydrylase (OASS) gene from a hyperthermophilic archaeon *Aeropyrum pernix*, which shares the pyridoxal 5′-phosphate binding motif with both OASS and cystathionine β-synthase (CBS) was cloned and expressed using *E. coli*. The purified enzyme catalyzes a cysteine synthetic reaction from O-acetyl-L-serine and sulfide (1). The enzyme retained 90% of its activity after 6-h incubation at 100°C. However, O-acetyl-L-serine is labile at high temperatures in which hyperthermophilic archaea live. Heat-stable O-phospho-L-serine was found to be reacted with the enzyme in a PLP-dependent manner to produce L-cysteine. The enzyme exhibited the highest activity for production of cysteine from O-phospho-L-serine (2). Kinetic analyses revealed that the O-phospho-L-serine sulfhydrylation reaction as well as the O-acetyl-L-serine sulfhydrylation reaction by the enzyme followed a ping-pong bi-bi mechanism. This enzyme has been identified as a new enzyme and named O-phosphoserine sulfhydrylase (OPSS; EC 2.5.1.65). The crystal structure of the enzyme was determined at 2.0 Å resolution using the method of multiwavelength anomalous dispersion (3, 4). A monomer consists of three domains, including an N-terminal domain with a new α/β fold. The topology folds of the middle and C-terminal domains were similar to those of the O-acetylserine sulfhydrylase-A from *Salmonella typhimurium* and the cystathionine β-synthase from human. The cofactor, pyridoxal 5′-phosphate, is bound in a cleft between the middle and C-terminal domains through a covalent linkage to Lys127. An enzyme-substrate complex model and a mutation experiment revealed that Arg297, unique to hyperthermophilic archaea, is one of the most crucial residues for O-phosphoserine sulfhydrylation activity. The reactivity of O-phospho-L-serine in the L-cysteine synthetic reaction provides a key for understanding the biosynthesis of L-cysteine by hyperthermophilic archaea.