Complementation of E. coli cysQ Mutant with Arabidopsis AHL Gene Encoding a 3'(2'),5'-Bisphosphate Nucleotidase

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Arabidopsis AHL gene encodes a 3'(2')-phosphoadenosine 5'-phosphate (PAP)-specific phosphatase that plays a role in the sulfate activation pathway. We complemented E. coli cysQ mutant defective in cysteine biosynthesis with the AHL gene. AHL cDNA was cloned into the prokaryotic expression vector pKK388-1 and transformed into the bacterial mutant. Since cysQ mutant is a leaky cysteine auxotroph only under aerobic conditions, the bacteria were grown in liquid media with vigorous shaking to provide more aeration. In cysteine-free medium, cysQ mutant and the mutant harboring empty vector did not grow well, whereas cells harboring AHL cDNA exhibited significantly improved growth with doubling time of approximately 3 h. cysQ is known to encode a 3'(2'),5'-bisphosphonucleoside 3'(2')-phosphohydrolase (DPNPase). However, our data suggest that cysQ protein has PAP-specific phosphatase activity in addition to DPNPase activity. Microbial complementation procedure described in this paper is useful for structure-activity studies of PAP-specific phosphatases identified from microbes and plants.

Key words: Arabidopsis AHL gene, E. coli cysQ mutant, DPNPase, PAP-phosphatase, cysteine-rich defense proteins, reductive sulfate assimilation.

Sulfur is one of the major elements of sulfur-containing amino acids, sulfated polysaccharides, sulfolipids, and coenzymes. In cellular sulfur metabolism, cysteine lies on the major route of inorganic sulfur incorporation into proteins and cysteine-rich peptides. Among the cysteine-rich peptides, glutathione and phytochelatins are the most abundant thiols compounds that play important roles in the detoxification of herbicides in plants. In addition, several families of small cysteine-rich proteins such as thionins and defensins exert antimicrobial activity in plant-pathogen interactions. Thus, sulfur metabolism in plants is closely related to various defense mechanisms against abiotic and biotic stresses.

Inorganic sulfate uptake from environment is chemically inert in living cells under moderate biological conditions. Thus, sulfate must be activated by reduction to sulfide before incorporated into sulfur-containing compounds. In E. coli and plants, sulfate is activated via coupling with ATP by ATP sulfurylase to form adenosine 5'-phosphosulfate (APS). A second ATP-coupled activation is conducted by APS kinase that converts APS into 3'-phosphoadenosine 5'-phosphosulfate (PAPS). PAPS reductase then reduces PAPS into sulfide that is further reduced into sulfate by sulfite reductase.

PAP, at high concentrations, exerts toxicity on cells. 3'(2'), 5'-Diphosphonucleoside 3'(2')-phosphohydrolase (DPNPase) has been suggested to catalyze the conversion of PAPS back into APS to avoid accumulation of PAPS. Proteins encoded by E. coli gene cysQ and rice RHL have been found to have DPNPase activity.

When PAPS is reduced into sulfate, 3'-phosphoadenosine 5'-phosphate (PAP) is generated as a by-product. PAP is then hydrolyzed into AMP and inorganic phosphate by a PAP-specific phosphatase, 3'(2'),5'-bisphosphate nucleotidase. Studies have found that the enzymes encoded by yeast HAL2 and Arabidopsis SAL1 and Arabidopsis AHL genes are PAP-phosphatases. The enzyme contributes to the rapid sulfur flux through the assimilation pathway by accelerating the PAPS-utilizing reactions. More importantly, the enzyme has been suggested to control the level of toxic PAP.

HAL2, SAL1, RHL, cysQ, and AHL have similar amino acid sequences including two motifs that are conserved in the inositol monophosphatase family. AHL, RHL, and SAL1 cDNAs complemented the yeast HAL2 mutant, met22. Moreover, the gene products of AHL, SAL1, RHL, and SAL1 utilize both PAPS and PAP as substrates in vitro. Thus, it is ambiguous whether these proteins belong to the PAP-phosphatase or DPNPase enzyme class.

Bacterial mutant complementation is one of the simplest procedures to demonstrate the enzyme activity of a protein encoded by a particular gene. However, complementation of...
the E. coli cysQ mutant has been hampered by the fact that the bacterial mutant is a leaky cysteine auxotroph only during aerobic growth. In this study, attempt was made to complement the E. coli cysQ mutant with the AHL gene by supplying more aeration to the culture.

Materials and Methods

Microbial strain. The E. coli mutant cysQ 5649 (cysQ::kan in David Botstein's 6128 [HfrH lacZ(Am), trp(Am) supII]) was kindly provided by Dr. Douglas E. Berg (Washington University School of Medicine, St. Louis, Missouri, USA).

DNA construct and transformation. The Arabidopsis AHL cDNA fragment was prepared by the polymerase chain reaction (PCR) using a high fidelity Taq polymerase (Boeringer Mannheim). The AHL cDNA was cloned into the prokaryotic expression vector pKK388-1 (Clontech Inc.) between the NcoI and EcoRI sites. The cysQ mutant cells were transformed with the construct or vector by electroporation. Transformed cells were then selected on LB plates containing 50 µg/ml each ampicillin and kanamycin. Orientation of the insert was examined by diagnostic restriction enzyme digestions and DNA sequencing.

Complementation experiments. The selected colony was grown in a liquid LB medium, and 5 ml saturated suspension was centrifuged at 3,800 rpm for 5 min. Subsequently, the bacterial residue was resuspended in 5 ml sterile water (OD₆₀₀ = ~2.0, ~1 × 10⁷/ml). Fifty microliters of the suspension was added to 50 ml liquid M9 minimal medium (NH₄Cl and CaCl₂ included) containing 1 M MgSO₄ and 0.2% glucose. The medium was supplemented with kanamycin (50 µg/ml), tryptophan (50 µg/ml), and 1 mM IPTG. For the control experiment, 50 µg/ml L-cysteine was added to the medium. The bacteria were grown in a rotary shaker (300 rpm) at 37°C, and their growths were determined by measuring the absorbances of the cultures at 600 nm. For solid medium, 1.5% Bacto agar was added to the medium. The bacteria were grown at 37°C for 2 days on the solid medium.

Results

Amino acid sequence homology. The E. coli cysQ (GeneID 1037415) and Arabidopsis AHL (GenBank AF016644) amino acid sequences were aligned using the cluster W program (Fig. 2). The BoxShade program was used to highlight identical amino acid residues. The two motifs that are conserved in the inositol monophosphatase family were found in both sequences. The two putative proteins exhibited 29.9% identity and 50.0% similarity in amino acid residues. Comparison of the nucleotide sequences revealed 39.0% homology between the cysQ and AHL genes (data not shown).
cysQ complementation on solid medium. To determine whether the protein product of AHL gene has a function similar to that of E. coli cysQ protein, complementation experiments with bacterial mutants were conducted. When each saturated culture was grown on cysteine-free solid medium (1.5% bacto-agar), the cysQ mutant cells harboring AHL cDNA grew slightly faster than the mutant cells harboring no or empty vector (Fig. 3). The growth of all strains, however, was indistinguishable after 2-3 days (data not shown). Thus, solid medium appears unable to maintain conditions sufficiently aerobic for growth repression of the cysQ mutant cells. A previous study revealed that the cysQ mutant exhibits leaky cysteine auxotrophic growth only under aerobic conditions. 6

cysQ complementation in liquid medium. To provide more aeration, the bacteria were grown in liquid media with vigorous shaking. Under these conditions, the cysQ mutant and cysQ mutant harboring empty vector did not grow well without the addition of L-cysteine (Fig. 4). In contrast, the cells harboring AHL cDNA exhibited significantly improved growth in the cysteine-free media with a doubling time of approximately 3 h. The newly devised complementation procedure is useful for the structure-function study of the cysQ-like proteins identified from a variety of living organisms.

Discussion

In this study, the bacterial cysQ mutant was successfully complemented with the Arabidopsis AHL gene. Solid medium was not adequate for the cysQ complementation experiments that require sufficiently aerobic condition for repression of the cysQ mutant cell growth. On the other hand, vigorous shaking of the liquid medium provided more aeration. The bacterial cells harboring AHL cDNA exhibited significantly improved growth in the cysteine-free medium with a doubling time of approximately 3 h. The newly devised complementation procedure is useful for the structure-function study of the cysQ-like proteins identified from a variety of living organisms.

Previous studies showed that E. coli gene cysQ encodes a 3’(2’)-5’-diphosphonucleoside 3’(2’)-phosphohydrolase (DPNPase) that catalyzes the conversion of 3’-phosphoadenosine 5’-phosphosulfate (PAPS) into adenosine 5’-phosphosulfate (APS) in the sulfate activation pathway. 9 In another set of experiments, AHL protein was identified to be a 3’(2’)-phosphoadenosine 5’-phosphate (PAP)-specific phosphatase. 4,7 Whereas HAL2, SAL1, and RHL proteins showed the same or higher activity with PAPS compared to PAP, 4,7 the AHL enzyme strongly prefers PAP as the substrate. 4,7 Thus, the AHL-complementation of cysQ mutant indicates that cysQ protein is also a PAP-specific phosphatase, without excluding the possibility that the protein has a DPNPase activity.

PAP-specific phosphatase encoded by the AHL gene contributes to the rapid sulfur flux in reductive sulfate assimilation of plants by eliminating PAP, as suggested by studies of similar enzymes in microbial systems. Furthermore, AHL enzyme is one of the initial targets for metal toxicity in plants. 41 Metal sensitivity of the HAL2 nucleotidase is an important determinant of yeast growth at high concentrations of Li+ and Na+. 12,31 Indeed, overexpression of hal2 significantly improved yeast growth at high salt concentrations. 35

In addition, the PAP-specific phosphatase enzyme activity controls the supply of cysteine, which is a main component of cysteine-rich peptides such as glutathione and phytochelatins that are involved in heavy metal-tolerance of plants. 3,4,14,20 Moreover, the plant defense proteins such as thionine and defensin are highly cysteine-rich. 52 Thus, sulfur metabolism
in plants is closely related to various defense mechanisms against abiotic and biotic stresses. Results of this study imply the possibility of generating plants tolerant to high salts or invading pathogens by overexpression of the PAP-specific phosphatase genes, such as cysQ and AHL.

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References


