Cysteine Sulfinate Desulfinase, a NIFS-like Protein of Escherichia coli with Selenocysteine Lyase and Cysteine Desulfurase Activities: Gene Cloning, Purification and Characterization of a Novel Pyridoxal Enzyme

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Cysteine Sulfinate Desulfinase, a NIFS-like Protein of *Escherichia coli* with Selenocysteine Lyase and Cysteine Desulfurase Activities: Gene Cloning, Purification and Characterization of a Novel Pyridoxal Enzyme

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Selenocysteine lyase (EC 4.4.1.16) exclusively decomposes selenocysteine to alanine and elemental selenium whereas cysteine desulfurase (NIFS protein) of *Azotobacter vinelandii* acts indiscriminately on both cysteine and selenocysteine to produce elemental sulfur and selenium respectively, and alanine. These proteins exhibit some sequence homology. The *Escherichia coli* genome contains three genes with sequence homology to *nifS*. We have cloned the gene mapped at 63.4 min in the chromosome, and have expressed, purified to homogeneity, and characterized the gene product. The enzyme comprises two identical subunits with 401 amino acid residues (Mr 43,238) and contains pyridoxal 5'-phosphate as a coenzyme. The enzyme catalyzes the removal of elemental sulfur and selenium atoms from L-cysteine, L-cystine, L-selenocysteine and L-selenocystine to produce L-alanine. Because L-cysteine sulfinic acid was desulfinated to form L-alanine as the preferred substrate, we have named this new enzyme cysteine sulfinate desulfinase. Mutant enzymes having alanine substituted for each of the four cysteinyl residues were all active. Cys358 corresponds to Cys325 of *A. vinelandii* NIFS, which is conserved among all NIFS-like proteins and catalytically essential is not required for cysteine sulfinate desulfinase. Thus, the enzyme is distinct from *A. vinelandii* NIFS in this respect.

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cysteine and L-selenocysteine indiscriminately. The enzyme was named cysteine desulfurase, based on its inherent physiological role.

The nucleotide sequence of the whole Escherichia coli genome has been determined (4), and the bacterium appears to contain three nifS-like genes. One of the genes located at 57.3 min in the chromosome presumably encodes the NIFS-like protein purified by Flint (5). Not only the amino acid sequence but also the catalytic properties of the enzyme resemble those of A. vinelandii NIFS. We have found that the N-terminal amino acid sequence of pig liver selenocysteine lyase is similar to that of A. vinelandii NIFS (6). If we assume that E. coli contains selenocysteine lyase and that the enzyme resembles NIFS, one or both of the other two nifS-like genes may encode selenocysteine lyase(s). Alternatively, the genes may encode new enzymes participating in an unknown metabolism of sulfur or selenium amino acids. We have cloned the nifS-like gene mapped at 63.4 min by PCR with the E. coli JM109 chromosomal DNA as a template, and investigated the properties of the gene product.

The molecular weight of the homogeneous preparation of the gene product estimated by SDS-PAGE (about 43,000) agreed with the value calculated from the deduced amino acid sequence (43,238). The molecular weight of the purified protein in the native form was estimated to be about 97,000 by gel filtration.

The enzyme showed at pH 7.4 an absorption maximum at 420 nm. Reduction with sodium borohydride resulted in the disappearance of the absorption band at 420 nm with a concomitant increase in the absorbance at 335 nm. The reduced enzyme was catalytically inactive. These results show that the enzyme requires pyridoxal phosphate as a cofactor.

The enzyme resembles selenocysteine lyase and NIFS in that it removes elemental sulfur or selenium from L-cysteine or L-selenocysteine in the reaction. L-Cysteine sulfinic acid acted as the best substrate of the enzyme, and essentially the same amounts of L-alanine and sulfite were produced in the reaction. Maximum activity for the desulfination was found at around pH 8.2 in Tricine-NaOH. We named our new enzyme cysteine sulfinate desulfinase, Cloning and expression of the Eco2 homologous gene, the last nifS-like gene of E. coli mapped at 38.3 min in the chromosome, and characterization of the gene product, are now being studied.

**References**