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 *nif*S. 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.

Keywords : Cysteine sulfinate desulfinase/ NIFS/ Pyridoxal phosphate

We found selenocysteine lyase in mammals and bacteria, and purified the enzyme from pig liver (1) and *Citrobacter freundii* (2). The enzyme specifically decomposes L-selenocysteine into L-alanine and elemental selenium; L-cysteine is inert as a substrate. Zheng *et al.* (3) recently demonstrated the function of NIFS protein, which is required for the efficient construction of the Fe-S clusters of nitrogenase in a diazotrophic bacterium *Azotobacter vinelandii*. NIFS catalyzes the same type of reaction as selenocysteine lyase, but acts on both L-

MOLECULAR BIOFUNCTION - Molecular Microbial Science -

Scope of research

Structure and function of biocatalysis, in particular, pyridoxal enzymes, NAD enzymes, and enzymes acting on xenobiotic compounds are studied to elucidate the dynamic aspects of the fine mechanism for their catalysis in the light of recent advances in gene technology, protein engineering and crystallography. In addition, the metabolism and biofunction of selenium and some other trace elements are investigated. Development and application of new biomolecular functions of microorganisms are also studied to open the door to new fields of biotechnology. For example, molecular structures and functions of thermostable enzymes and their application are under investigation.

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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 *nif*S-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 *nif*S-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 *nif*S-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, because the enzyme showed the lowest K_m value and the highest k_{cat} and k_{cat}/K_m values for L-cysteine sulfinic acid .

NIFS has been classified into the same group as aminotransferases of class V (7) and subgroup IV (8), which include serine-pyruvate aminotransferase (EC 2.6.1.51) and phosphoserine aminotransferase (EC 2.6.1.52), on the basis of sequence homology analysis. We have found that NIFS family proteins are classified into two groups, I and II, according to their sequence similarities. Average sequence similarities of cysteine sulfinate desulfinase to Group I and II members were 23 and 37%, respectively.

NIFS of *A. vinelandii* participates in construction of the Fe-S clusters of not only nitrogenase, but also other iron-sulfur proteins such as SoxR and FNR. The NIFS-like enzyme of *E. coli* found by Flint (5) also provides apo dihydroxy-acid dehydratase with a [4Fe-4S] cluster to reconstitute the enzyme *in vitro*. NIFS and the NIFS-like protein from *E. coli*, which belong to Group I, have common characteristics: both contain essential cysteinyl residues at the active sites. The thiol group presumably attacks as a nucleophile the sulfur atom of the substrate, cysteine, to form the intermediate, enzyme-bound cysteinyl persulfide (3, 5). By contrast, no cysteinyl residue of cysteine sulfinate desulfinase is essential for catalysis. The cysteine sulfinate desulfinase-reaction is assumed to proceed through direct release of elemental selenium or sulfur atom from the substrate, selenocysteine or cysteine. It has been assumed that formation of the enzyme-bound cysteinyl persulfide is crucial to deliver sulfur atoms efficiently to iron-sulfur proteins. If this is the case, cysteine sulfinate desulfinase will not be related metabolically to the formation of Fe-S clusters, although sulfur atoms produced from cysteine by the enzyme are probably incorporated into iron-sulfur proteins with low efficiency. The fact that the K_m value of cysteine sulfinate desulfinase for cysteine is high also suggests that cysteine is not the physiological substrate of the enzyme. Whatever the physiological function of cysteine sulfinate desulfinase is, this is the first enzyme in Group II whose catalytic function has been clarified. Other proteins of this group probably have a similar catalytic function to cysteine sulfinate desulfinase. Cloning and expression of the Eco2 gene, the last *nif*S-like gene of *E. coli* mapped at 38.3 min in the chromosome, and characterization of the gene product, are now being studied.

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