Cystathionine β-Synthase: Structure, Function, Regulation, and Location of Homocystinuria-causing Mutations*

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Cystathionine β-synthase (CBS)1 (EC 4.2.1.22) catalyzes the pyridoxal 5'-phosphate (PLP)-dependent β-replacement reaction in which the thiolate of L-homocysteine replaces the hydroxyl group of L-serine (Equation 1).

\[
\text{HOC}_{2}\text{H}_{4}\text{CH(NH}_{2}\text{)}_{2}\text{COOH} + \text{HSCH}_{2}\text{CH_{2}}\text{CH(NH}_{2}\text{)}_{2}\text{COOH} \rightarrow \text{CH}_{2}\text{C}_{\text{CBS2}}\text{CH(NH}_{2}\text{)}_{2}\text{COOH} + \text{H}_{2}\text{O}
\]

L-Serine + L-Homocysteine \rightarrow L-Cystathionine

(Eq. 1)

Human CBS is an especially interesting PLP enzyme because it has a complex domain structure (Fig. 1) and regulatory mechanism. The allosteric activator, S-adenosyl-L-methionine (AdoMet), increases CBS activity about 3-fold (1) and likely binds to the C-terminal regulatory domain (2). CBS from higher eukaryotes contains a unique heme moiety of unknown function (3–5), which is not found in CBS from yeast (Saccharomyces cerevisiae) (6–8) or from the protozoan hemoflagellate, Trypanosoma cruzi (9).

A large number of mutations in different regions of the human CBS have been found in patients with homocystinuria, a human hereditary disease that is characterized by very high plasma levels of the toxic amino acid L-homocysteine (10, 11). The clinical phenotype of patients with homocystinuria includes mental retardation, lens dislocation, skeletal abnormalities, and vascular disease (10). Mutations in the CBS gene can alter either mRNA or enzyme stability, activity, binding of PLP and heme, or impair allosteric regulation.

Crystal structures of truncated forms of the human enzyme have revealed the structure of the catalytic domain of and the N-terminal heme-binding site (12, 13). The location of homocystinuria-causing mutations in the three-dimensional structure of human CBS is of interest, although the roles of the mutated residues are not fully understood (12, 14).

This minireview focuses on relationships between CBS and other PLP enzymes, the structure, function, and regulation of CBS, and the relation of the structure and function of CBS to homocystinuria.

Relationships between Cystathionine β-Synthase and Other Pyridoxal Phosphate Enzymes

Enzymes that have a PLP coenzyme catalyze a wide variety of reactions in amino acid metabolism (15). PLP enzymes are divided into four families on the basis of similarities in three-dimensional structure, sequence, secondary structure, and hydrophobicity profiles (15, 16). Aspartate aminotransferase is the prototype of the largest family, the α family (15) or Fold type I (16). The tryptophan synthase β subunit is the prototype of the second largest family, the β family or Fold type II, which also contains CBS. Fig. 2 shows members of the β family and their evolutionary pedigree (15). CBS is most closely related to O-acetylserine sulfhydrolase (cysteine synthase). The close structural relationship between the catalytic domains of these two enzymes has been demonstrated by x-ray crystallography (12, 13).

Protein Domains in Cystathionine β-Synthase and in Other β Family Enzymes

The alignment of several β family chains at the active site lysine that binds PLP (Fig. 3) shows that O-acetylserine sulfhydrolase (322 residues) represents the simplest catalytic core of enzymes in the β family and that most of the other enzymes have N- or C-terminal extensions that may serve regulatory functions.

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‡ The abbreviations used are: CBS, cystathionine β-synthase; PLP, pyridoxal 5'-phosphate; AdoMet, S-adenosyl-l-methionine.

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**Fig. 1.** The modular domain structure of human CBS showing the N-terminal domain that binds heme, the catalytic domain, and the C-terminal regulatory domain that contains two “CBS” domains, CBS1 and CBS2. We thank Jana Oliveriusova for preparing this figure.

**Fig. 2.** Members of the β family of PLP enzymes and their evolutionary pedigree. Reprinted from Ref. 15 with permission.
roles. Biosynthetic threonine deaminase from \textit{Escherichia coli} has a C-terminal extension that binds a feedback inhibitor (17). The structure of threonine deaminase reveals that the C-terminal regulatory domain projects out from a core of the catalytic PLP-containing N-terminal domain (17). Protein sequence alignments show that members of the \( \beta \) family exhibit significant homology in the core, catalytic region and little similarity in the N- and C-terminal extensions.

A regulatory role for the C-terminal extension of human and yeast CBS is supported by the finding that removal of the C-terminal domain of the human enzyme (2, 18–20) or of the yeast enzyme (7) increases the specific activity and alters the steady-state kinetic parameters. AdoMet does not activate the truncated human enzyme. \textit{T. cruzi} CBS lacks the C-terminal domain and is not activated by AdoMet (9). Removal of the C-terminal domain causes human CBS (2) and yeast CBS (7) to dissociate from tetramers or higher multimers to dimers.

Human CBS contains an N-terminal region of ~70 amino acids (Figs. 1 and 3) that binds heme (12) and is absent in yeast CBS (6) and in \textit{T. cruzi} CBS (9), which do not contain heme. Analysis of the products of deletion mutagenesis of human CBS reveals that the N-terminal amino acids 1–39 do not play a significant role in structure or function (2, 20) (Fig. 3). Deletion of residues 1–70 yielded enzyme with reduced activity that did not bind heme; C-terminal truncation did not affect heme binding. Deletion of residues 1–70 and 401–551 yielded the catalytic core that had low activity and bound PLP but not heme (20). Sensitivity of CBS to AdoMet can be abolished by deleting eight residues from the C terminus but not just one residue (20).

The C-terminal regulatory region also encompasses the previously defined “CBS domain” (21). This hydrophobic sequence (CBS1), spanning amino acid residues 415–468 (Fig. 1), is conserved in a wide range of otherwise unrelated proteins (21) (www.sanger.ac.uk/Users/agb/CBS/CBS.html). Based on sequence similarity with another CBS domain containing protein, inosine-monophosphate dehydrogenase from \textit{Streptomyces pyogenes}, a second, less conserved CBS domain (CBS2) was identified (22) between amino acid residues 486 and 543 in the C-terminal regulatory region of human CBS (Fig. 1).

The function of these domains in human CBS remains unknown, although the sharp transition of thermally induced transitions of CBS activates the enzyme (23). Recent work demonstrates that the tandem pairs of CBS domains (CBS residues 416–551) bind AdoMet (24).

**Crystal Structures of Truncated Human Cystathionine \( \beta \)-Synthase**

Two groups have solved crystal structures of truncated forms of human CBS (12, 13). The structure of enzyme containing residues 1–413 (12) demonstrates that the fold of the catalytic domain closely resembles the catalytic domain of other \( \beta \) family structures: O-acetylseryne sulfhydrylase, threonine deaminase, aminocyclopropane deaminase, and threonine synthase. Heme binds to the N-terminal region at distal ends of the dimer (Fig. 4). His-65 and Cys-52 are the ligands to the heme iron. Both structures reveal important details about the PLP-binding site and residues in the catalytic site.

**Catalytic Mechanism of Cystathionine \( \beta \)-Synthase**

Although the spectroscopic properties of PLP provide a sensitive probe for detecting intermediates in the CBS reaction, the presence of heme in human CBS largely masks the spectrum of PLP (25). Thus, the heme-independent yeast CBS is a useful substitute for studies of catalytic mechanism and kinetics (6–8, 26–29). Addition of \( L \)-serine to yeast CBS results in the disappearance of the 412 nm band of CBS and the appearance of a new species (\( \lambda_{\text{max}} = 460 \) nm), which is attributed to a PLP-aminoacylate intermediate (6). This intermediate is first observed in the closely related enzyme, \( O \)-acetylseryne sulfhydrylase (30). Addition of \( l \)-cystathionine also yields an aminoacylate intermediate, demonstrating the partial reversibility of the reaction. Heme-free crystals of human CBS also convert \( l \)-serine to an aminoacylate intermediate as demonstrated by single crystal microspectrophotometry (31).

Investigations of the steady-state kinetics of yeast CBS (7) and human CBS (2, 32) have utilized a sensitive but tedious \( ^{14} \)C-labeled \( l \)-Ser assay. Kinetic data for truncated yeast CBS are consistent with a ping-pong mechanism in which aminoacylate is a key intermediate (7). Aitken and Kirsch (29) have used continuous assays for the forward and reverse reactions to study the kinetics of the truncated yeast CBS and to determine the equilibrium constant and the pH dependence of the kinetic parameters. The rate of the forward reaction is 38-fold greater than the reverse reaction. Thus, the CBS reaction strongly favors \( l \)-cystathionine formation \textit{in vivo}. Recent kinetic studies of mutants of yeast CBS have characterized the roles of puta-
Alternate Substrates and Reactions

CBS catalyzes PLP-dependent β-replacement reactions (Equation 2) in which the electrenegative substituent (X) in the β-position of the amino acid substrate is replaced by a nucleophile YH (reviewed in Refs. 34 and 35). β-Replacement reactions (Equation 2) are also catalyzed by tryptophan synthase, O-acetylserine sulfhydrylase, and several other PLP enzymes.

\[
XCH_2CH(NH_2)COOH + YH \leftrightarrow XH + YCH_2CH(NH_2)COOH \quad (\text{Eq. 2})
\]

where X is OH or SH and Y is S or S-alkyl.

Amino acid substrates for CBS include l-serine (X is OH), l-cysteine (X is SH), 3-chloroalanine (X is Cl), and serine O-sulfate (X is SO₄); nucleophile substrates (YH) include l-homocysteine, 2-mercaptoethanol, and H₂S (34, 35). The reaction of l-cysteine and 2-mercaptoethanol to form S-hydroxyethyl-l-cysteine and H₂S is the basis of useful assay methods (7, 36).

Recent studies provide evidence that H₂S is a gaseous neuromodulator and smooth muscle relaxant and that H₂S is produced by CBS (37). Although the author suggests that H₂S is produced by a β-elimination reaction with l-cysteine, H₂S may be a product of the β-replacement reaction of l-cysteine with another thiol (38). CBS will also very efficiently catalyze the formation of l-cysteine from L-serine and H₂S. This serine sulfhydrylase reaction may be an alternative method of cysteine synthesis and H₂S detoxification.²

1-Allothreonine, but not l-threonine, serves as a primary substrate for yeast CBS and reacts with l-homocysteine to form a new amino acid, 3-methyl-l-cystathionine (27). The reaction has been characterized by spectroscopic measurements under pre-steady-state and steady-state conditions.

Regulation of Cystathionine β-Synthase

The human CBS gene is transcriptionally regulated by two promoter regions designated −1a and −1b (39). The major promoter (~1b) is serum and fibroblast growth factor-responsive and is down-regulated by insulin, growth arrest due to contact inhibition, nutrient depletion, or the induction of differentiation (40). The CBS −1b promoter is regulated in a redox-sensitive fashion by synergistic interactions between Sp1 and NF-Y and Sp1 and Sp3. Sp1 and Sp3 are the specificity factors 1 and 3, respectively, whereas NF-Y is the nuclear factor Y, a histone-like CCAAT-binding trimer (reviewed in Refs. 41 and 42). The dominant and indispensable role of Sp1 in regulating both GC-rich CBS promoters may allow tissue-specific repression by Kruppel-like factors (43–45). Sp1-like proteins and Kruppel-like factors are highly related redox-sensitive zinc-finger proteins that are important components of the eukaryotic cellular transcriptional machinery.

In contrast to the relatively slow transcriptional response, AdoMet can instantaneously activate human CBS. Proteolytic removal of the C-terminal region also activates human CBS; the extent of activation is similar to that observed with AdoMet (2, 20, 46). Although the yeast enzyme is also activated by removal of the C-terminal domain, AdoMet does not activate the yeast enzyme and no other activator has been discovered (7).

The role of heme in human CBS is still not clear. Heme is not essential for catalysis because it is absent in yeast CBS (6–8) and T. cruzi CBS (9) and because heme-free human CBS has activity (20, 31). Human CBS may also be regulated by the redox state of the heme. Whereas one group observed a ~2-fold decrease in CBS activity upon reduction of heme (reviewed in Ref. 47), another group did not observe this change.²

Location of Homocystinuria-causing Mutations in Cystathionine β-Synthase

Mutations found in patients with homocystinuria are distributed widely in the catalytic and regulatory domains of human CBS (for a continuously updated list of more than 130 mutations, see www.uchsc.edu/sm/che/che) (11). About half of these mutations result in a B₉₇-responsive clinical phenotype, i.e. treatment with high doses of the PLP precursor, pyridoxine or vitamin B₉, is clinically effective. More than 20 years ago it was shown that cellular CBS activity can be increased by pyridoxine supplements in vivo with patients whose mutant enzyme had moderately reduced affinity for PLP but not with patients whose enzyme had a more dramatically reduced affinity for the coenzyme (48). These findings have recently been corroborated using a purified enzyme (49). The mutant enzyme, V168M, exhibited a ~7-fold decrease in bound PLP and a ~13-fold

² J. P. Kraus, unpublished data.
decrease in activity (49). V168M and a number of other patient-derived mutations in the catalytic domain are alleviated by deletion of the C-terminal regulatory domain (18, 49) or by specific point mutations in this region (22, 23, 50). Most of these point mutations are located in the CBS1 domain (21) (Fig. 1) and block or impair activation by AdoMet. The S466L mutant enzyme is constitutively activated; although this mutant enzyme is highly pyridoxine-responsive (9). The I278T mutant enzyme is in the active site, may be in the L-homocysteine-missing regulatory domain (Fig. 4). The two most prevalent interface, the active site, the heme-binding site, and the pre-truncation, partial thermal denaturation, and AdoMet all induce similar levels of activation of the wild type enzyme suggests that these different forms of activation are acting through a similar mechanism, possibly by displacing the inhibitory domain from the active site (23) (see Refs. 22, 47, and 50 for related models).

Analysis of the crystal structure of the dimeric truncated human CBS (residues 1–413) (12, 14) shows that disease-causing mutations are distributed in several areas: the dimer interface, the active site, the heme-binding site, and the predicted interface region between the catalytic domain and the missing regulatory domain (Fig. 4). The two most prevalent mutations in CBS-deficient patients are I278T, which is responsible for pyridoxine-insensitive activation, and G307S, which is not (11). G307 lines the entry to the active site, may be in the l-homocysteine-binding site, and likely plays an essential role (14). The residue Gly-305 contacts with the pyridine ring of PLP (Fig. 4). The G305R mutation is pyridoxine-responsive and probably weakens PLP binding. Ala-114 is in the dimer interface; the A114V mutant enzyme is highly pyridoxine-responsive (9). The I278T mutation is corrected by certain mutations in the C-terminal domain or by deletion of this domain (18, 22). The I278T mutation and many of the less prevalent mutations probably affect the conformation, the folding, or the stability of CBS. Future work will likely concentrate on solving the structure of the full-length enzyme to see how the regulatory region interacts with the catalytic core and how the human mutations disrupt this interaction.

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