

Secondary Structure of Recombinant Human Cystathionine β -Synthase in Aqueous Solution: Effect of Ligand Binding and Proteolytic Truncation¹

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The secondary structural composition and substrate-induced conformational changes of recombinant human cystathionine β -synthase (CBS) in aqueous solution have been investigated in its full-length form (tetramer of 63-kDa subunits) by Fourier transform infrared (FT-IR) and circular dichroism (CD) spectroscopies. In addition, structural comparison of a proteolytic truncated form (dimer of 45-kDa subunits) to that of the full-length enzyme has also been carried out. Second-derivative and Fourier self-deconvolution enhanced infrared spectra revealed amide I band components ascribed to β -sheet (1689, 1638, and 1627 cm^{-1}), α -helix (1658 cm^{-1}), β -turn (1679 and 1668 cm^{-1}), and unordered (1651 cm^{-1}) structures in the spectra of the full-length enzyme. Quantitative analysis of FT-IR and CD spectra reveals that the full-length enzyme consists of about 48–53% β -sheet, 25–30% α -helix, 8–10% turn, and 10–19% unordered structures. Under constraint of the spectroscopic data, theoretical prediction of locations of these secondary structural elements using Garnier's method shows that human CBS may contain a β -sheet/ α -helix/ β -sheet core structure. Second-derivative spectrum of the truncated enzyme exhibited all the major spectral features that are present in the full-length enzyme, indicating a preservation of the core structure of the enzyme. Significant differences were observed between the infrared spectra of the enzymes with or without the substrate, serine, indicating a substrate-induced conformational

change in the enzyme, which did not result in a change in overall composition of secondary structural content based on quantitative analysis of FT-IR and far-UV CD spectra. © 1997 Academic Press

Key Words: cystathionine β -synthase; secondary structure, FT-IR; CD.

Cystathionine β -synthase (L-serine hydrolyase, EC 4.2.1.22) (CBS)³ deficiency is the leading cause of homocystinuria in humans (1). CBS catalyzes the first step of homocysteine transsulfuration by conjugating homocysteine and serine to form cystathionine, which is subsequently converted into cysteine and α -ketobutyrate by cystathionine γ -lyase (1). Pyridoxal 5'-phosphate is a cofactor of CBS for these reactions (2), and S-adenosylmethionine an allosteric activator (3). Recently, cDNA encoding rat and human CBS have been cloned, and the amino acid sequences have been deduced (4, 5). Independent comparisons of inferred amino acid sequences of rat liver CBS and the so-called "hemoprotein H-450" from rat liver to O-acetylserine (thiol)-lyase from *Escherichia coli* have revealed a 52% sequence homology (4, 6). Subsequent direct comparison has revealed that the cDNA sequence of CBS was identical to that of hemoprotein H-450. Both rat and human liver enzymes contain oxidized (Fe^{3+}) heme *b* which appears to be essential for maintaining the active structure of the enzyme and its activity (7).

The mammalian CBS is a tetrameric protein with identical subunits of 63 kDa. Multiple forms of the

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³ Abbreviations used: CBS, cystathionine β -synthase; FT-IR, Fourier transform infrared; IR-SD, infrared second-derivative; CD, circular dichroism; FSD, Fourier self-deconvolution.

native enzyme from 240-kDa tetramers to large multimers have been isolated (8). Early observations showed that proteolysis of CBS in liver extract converts the native tetrameric enzyme of 63-kDa subunits into active dimers of 48-kDa subunits (9). A fully active dimeric form with 45-kDa truncated subunits has also been prepared from the full-length enzyme by proteolytic digestion with trypsin. Enzyme assay has shown that the specific activity of the truncated 45-kDa enzyme is twice as large as its parent enzyme.

Little is known about the secondary structural composition of either the full-length enzyme with 63-kDa subunits or the truncated form with 45-kDa subunits due to technical difficulties encountered during crystallization of the proteins. However, some progress has been made in the crystallization of truncated enzyme (Kery and Kraus, unpublished data). Considering the likelihood of crystallographic analysis of the crystallizable truncated dimer rather than the full-length form, it is important to make a structural comparison of these two forms of CBS. In the present study, we have analyzed comparatively the secondary structures of the full-length and truncated recombinant human CBS and their ligand-dependent conformational changes by FT-IR and CD spectroscopies.

MATERIALS AND METHODS

Cloning and expression of full-length human CBS. The full-length enzyme was prepared, isolated, and purified in a H₂O-based buffer as described previously (10). The expression vector pAX5⁻ (U.S. Biochemical Corp.) in *E. coli* XL-1 blue MR (Stratagene) were used. The enzyme was expressed with β -galactosidase as the fusion partner and precipitated from the crude cell lysate with ammonium sulfate. Human CBS was cut off from the fusion protein by endoproteinase Xa (USB Life Sciences Research Products) and purified to homogeneity by DE-52 column chromatography as described previously (10). The percentage of heme saturation was measured spectrophotometrically according to the method of Berry and Trumpower (11).

Truncated form of CBS. Truncated 45-kDa enzyme was obtained by trypsinolysis of the fusion protein β -galactosidase—human CBS for 30 min at 37°C in 10 mM Tris-HCl, pH 8.0. The w/w ratio of trypsin to the fusion protein was 1/100. Further purification of the truncated form was performed as described for the full-length enzyme.

Sample preparations. Hydrogen-deuterium exchange was carried out by dialyzing a concentrated CBS sample against 50 mM phosphate/D₂O (99.9 atom% D, Sigma) buffer (pH 8.6, direct reading). The dialysis buffer was replaced four times in 48 h. The final concentration of CBS was about 3 mg/mL. For the serine-binding experiment, a concentrated serine in D₂O solution was added to CBS sample, prior to FT-IR measurement, to a final concentration of 4 mM of serine.

Infrared measurement and spectral analysis. Infrared spectra of human CBS were measured at 25°C with a Magna-IR Model 550 spectrometer (Nicolet), equipped with a dTGS detector. CBS solutions for infrared measurements were loaded into a IR cell (Beckman FH-01) with CaF₂ windows and a 50- μ m path length. For each spectrum, a 512-scan interferogram was collected in single beam mode with a 4 cm⁻¹ resolution and Happ-Genzel apodization. Reference (buffer only) spectra were also recorded as a 512-scan interferogram under identical scan conditions. Protein spectra were obtained according to previously established subtraction criteria [criterion 1, the bands originating from

water vapor must be subtracted accurately from the protein spectrum in the 1800 to 1500 cm⁻¹ region regardless of the baseline (gaseous water subtraction) and criterion 2, a straight baseline must be obtained from 2000 to 1750 cm⁻¹ (liquid water subtraction)] and procedures (12, 13). The resultant spectrum was smoothed with a 7-point window Savitsky-Golay function to remove the white noise. Infrared second-derivative (IR-SD) spectra were obtained with the derivative function of Omnic software (Nicolet). The inverted second-derivative spectra were obtained by factoring by -1 and then curve-fitted as described previously (14). Fourier self-deconvolution (FSD) analysis was carried out by the method of Kauppinen and colleagues (15, 16) with a half-bandwidth (full-width at half-height) of 20 cm⁻¹ and an enhance factor (*K* value) of 2.7. The resultant spectrum was then curve-fitted by the method of Susi and Byler (17).

Circular dichroism measurement and spectral analysis. The CD spectra were measured with an Aviv 62DS Spectrometer (Lakewood, NJ), equipped with a thermoelectric temperature control unit. All temperatures were regulated to within 0.1°C. Spectra were recorded at 25°C with 1-mm quartz cells. The protein concentration was 0.1 mg/mL, in 50 mM phosphate/D₂O buffer (pH 8.6, direct reading). Background spectra were recorded under identical experiment conditions. The difference spectrum was generated by subtracting the background spectrum from the corresponding protein spectrum. The spectra were deconvoluted using PROSEC program described previously (18).

Theoretical analysis of cystathionine β -synthase secondary structure. Theoretical analysis of the secondary structure of the full-length CBS was carried out using previously described algorithms (19) with the aid of PC-Gene software (Intelligenetics, Mountain View, CA) at Colorado State University. The decision constants employed in the Garnier secondary structure prediction for α -helix, β -sheet, turn, and unordered structures were -30, -125, 0, and 0, respectively.

RESULTS

Assignments of amide I band components. Figure 1 presents the infrared spectrum of the full-length CBS (oxidized form) in 50 mM phosphate/D₂O buffer, together with its FSD and IR-SD spectra. The smoothness and featurelessness of the nonstructural region between 1900 and 1720 cm⁻¹ in the IR-SD spectrum indicates a successful elimination of the spectral contributions of water vapor, which can be present in the infrared radiation path even under extensive dry air purging (13). The full-length enzyme exhibits an amide I band maximum near 1640 cm⁻¹ indicating a predominantly β -sheet structure (12-14, 17, 20).

Two infrared band contours assignable to the amide II and amide II' vibrations are observed near 1569 and 1455 cm⁻¹, respectively. The amide II band arises mainly from an out-of-phase combination of N-H in-plane bending and C-N stretching vibrations of peptide linkages (21) and is sensitive to the exchange of hydrogen to deuterium on protein backbone (17, 20, 22). The amide II' band at 1455 cm⁻¹ is the isotopic product of H-D exchange of the amide II band. The coappearance of the amide II and amide II' bands is apparently a result of incomplete H-D exchange on the protein backbone.

Both FSD and IR-SD analyses reveal similar amide I spectral patterns, in which six band components near

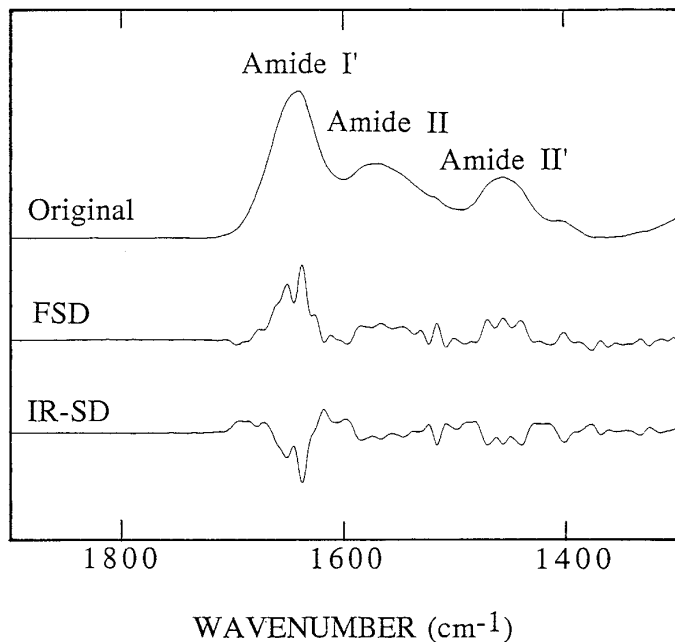


FIG. 1. Infrared spectra of oxidized human CBS in 50 mM phosphate/D₂O buffer (pH 8.6). The amide I', amide II, and amide II' bands are observed near 1640, 1569, and 1455 cm⁻¹, respectively. The Fourier self-deconvoluted and second-derivative spectra were obtained from the original spectrum as described under Materials and Methods.

1689, 1679, 1658, 1651, 1638, and 1627 cm⁻¹ are visually identifiable. An additional band component near 1668 cm⁻¹ is revealed by the curve-fitting analysis (Fig. 2). On the basis of earlier infrared studies of other proteins (12–14, 17, 20), the band components at 1689, 1638, and 1627 cm⁻¹ can be assigned to β -sheet, the band near 1658 cm⁻¹ to α -helix that is not affected by H–D exchange, and the bands at 1679 and 1668 cm⁻¹ to β -turn structure.

Assignment of the 1651 cm⁻¹ band is complicated by the fact of incomplete H–D exchange at backbone of the CBS. It has been known that the frequency of amide I component arising from an α -helical structure may downshift about 6 cm⁻¹ from about 1656 cm⁻¹ in H₂O to near 1650 cm⁻¹, when the protein is transferred into a D₂O solution (17, 20). Under this circumstance, the α -helical band could overlap with the band arising from unordered structure, which locates at 1648 ± 2 cm⁻¹ in H₂O (12, 13) and 1645 ± 4 cm⁻¹ in D₂O (17, 20). Thus, the band component near 1651 cm⁻¹, in this case, is better assigned to a combination of α -helical structure affected by H–D exchange and unordered structures. The weak bands below 1620 cm⁻¹ have been generally assigned to the side-chain vibrations of amino acid residues (23).

Truncated enzyme. Figure 3 presents an overlay of second-derivative spectra of the truncated 45-kDa enzyme and its full-length 63-kDa parent in the amide I

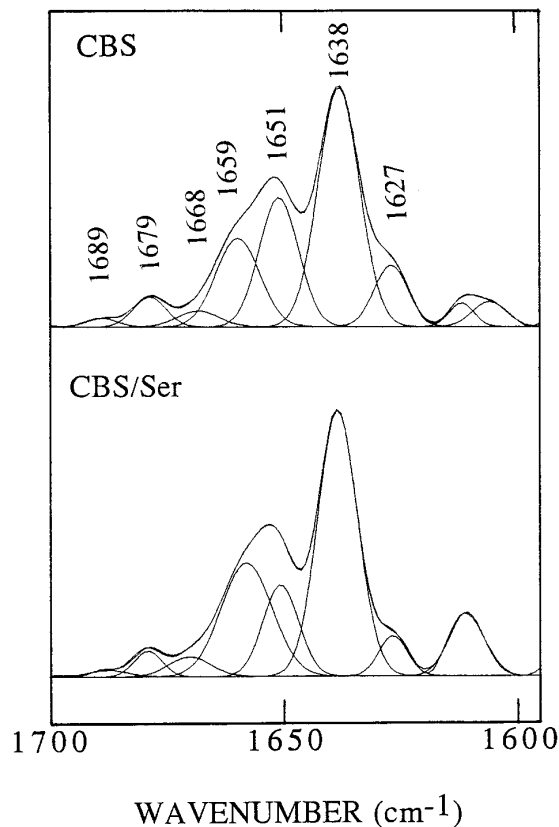


FIG. 2. The curve-fitting of inverted second-derivative spectra of the full-length CBS with or without 4 mM serine. The curve-fitting procedure was carried out as described under Materials and Methods, and the resultant parameters are listed in Table II.

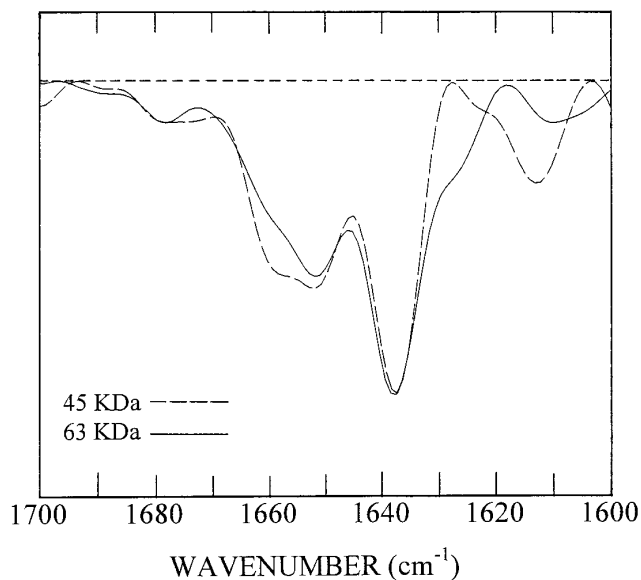


FIG. 3. Comparisons of the second-derivative spectra of the full-length 63-kDa and truncated 45-kDa enzymes. Solid line, full-length 63-kDa enzyme; broken line, truncated 45-kDa enzyme. The two spectra are normalized to the same intensity at their maxima for easier comparison.

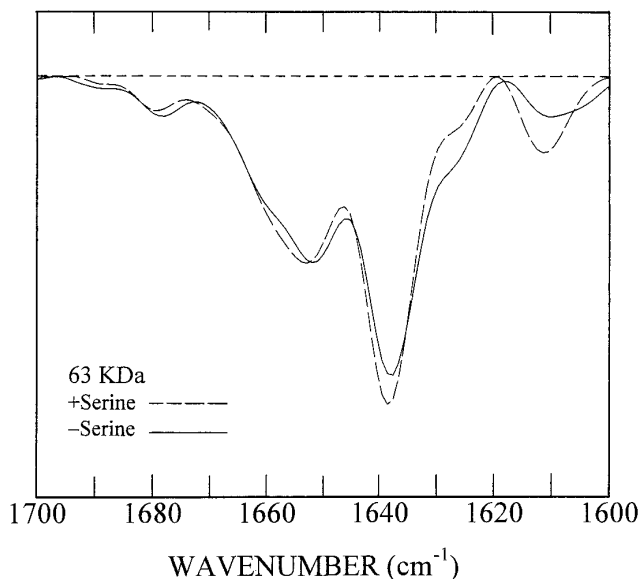


FIG. 4. Comparisons of the second-derivative spectra of the full-length CBS with or without 4 mM serine. Solid line, CBS without serine; broken line, with 4 mM serine.

region. The two spectra have been normalized to the same intensity at their maxima for easier comparison. We like to point out that a direct spectral comparison between the truncated 45-kDa dimeric form and its full-length 63-kDa tetrameric parent can be misleading due to the obvious differences in their molecular weight and association patterns. However, important information can be obtained from qualitative comparison focusing on the major spectral patterns. The second-derivative spectrum of the truncated enzyme shows all major amide I band components that are present in the spectra of the full-length enzyme with the exception of the band component near 1627 cm^{-1} . This result suggests that the core structure of CBS is preserved well in the truncated enzyme, which is consistent with the observation of a full enzymatic function of the truncated enzyme.

Serine-induced changes in conformational dynamics of CBS. To see whether substrate-binding affects the conformation of the enzyme, we carried out detailed spectral comparisons, quantitatively as well as qualitatively, of the full-length CBS in the presence and absence of 4 mM serine (Figs. 2 and 4). For quantitative analysis, the curve-fitting procedure was performed on both the FSD spectra by the method of Susi and Byler (17, 20), and the inverted IR-SD spectra by the method of Dong *et al.* (14). The overall secondary structures of CBS estimated from the two independent quantitative methods are summarized in Table I. Only the inverted second-derivative spectra with curve-fitting are shown in Fig. 2 and the resultant parameters for individual components are listed in Table II. Serine absorptional

contribution has been subtracted from the spectrum of CBS-serine complex.

Serine binding does not alter the overall secondary structural composition of the enzyme (Table I). Human CBS, with or without bound serine, consists of 50–53% β -sheet, 38–40% α -helix/unordered structure combination, and 7–10% turn. However, significant spectral differences in regions ascribed to α -helix, unordered, and β -sheet structures were observed, indicating a substrate-induced conformational change in the enzyme. Since the enzyme used in the experiment was deuterated prior to serine binding, spectral changes in the conformationally sensitive amide I region observed after addition of serine should result from the enzyme-substrate interaction, not from the changes in enzyme deuteration. The specificity of the serine-dependent changes was confirmed in a control experiment when serine was substituted by threonine, in which no spectral changes were observed (data not shown). The absorption redistribution between two bands at 1658 and 1627 cm^{-1} upon serine binding suggests that the substrate-induced conformational changes involves a spatial rearrangement of α -helices between the two states. The larger band area under 1658 cm^{-1} in the serine-bound state may result from stronger interactions among α -helices.

Far-UV CD spectra. Figure 5 shows the far-UV CD spectra of the truncated 45-kDa enzyme and its full-length 63-kDa parent. Figure 6 shows the far-UV CD spectra of full-length CBS with or without 4 mM serine. The far UV (180–250 nm) CD spectra, which indicate the overall secondary structural composition of the two proteins, suggest there are similar structures for the two CBS forms. The intensity at 222 nm is slightly greater for the 63-kDa protein, usually indicating a

TABLE I

Secondary Structural Composition of Full-Length Human Cystathionine β -Synthase as Estimated by Infrared and Circular Dichroism

State	Secondary structure (%)			Method
	α -Helix and U ^a	β -Sheet	Turn	
No serine	39.3	53.2	7.5	IR-SD ^b
	40.3	48.1	11.6	FSD ^c
	α 25/U19	50	6	CD ^d
With 4 mM serine	39.7	52.9	7.4	IR-SD
	39.1	48.0	12.9	FSD
	α 25/U19	50	6	CD

^a U, unordered, due to effect of H-D exchange, the contents of α -helix and unordered structures cannot be individually determined by infrared methods.

^b Infrared second-derivative, the method by Dong *et al.* (14).

^c Fourier self-deconvolution, the method by Kauppinen *et al.* (15, 16) and Susi and Byler (17).

^d Deconvoluted using PROSEC, the method of Yang *et al.* (18).

TABLE II
Assignments and Relative Areas of Deconvoluted Infrared Amide I Components of Full-Length Human Cystathionine β -Synthase in Aqueous Solution^a

ν (cm ⁻¹)	CBS		ν (cm ⁻¹)	CBS + serine		Assignment
	Area (%)			Area (%)		
	IR-SD	FSD		IR-SD	FSD	
1627	8.9	9.8	1627	4.9	7.6	β -sheet
1638	43.1	37.2	1638	46.8	39.0	β -sheet
1651	22.2	23.4	1651	14.1	14.3	α -helix + unordered
1659	17.1	16.9	1658	25.6	24.8	α -helix
1668	3.1	4.4	1670	3.8	5.3	turn
1679	4.4	7.2	1679	3.6	7.6	turn
1689	1.2	1.1	1688	1.2	1.4	β -sheet

^aThe band components below 1620 cm⁻¹, which arise from side chain vibration, are not included in calculation.

higher helix content. However, the crossover wavelength for the 45-kDa fragment is blue-shifted and the intensity at 195 nm is higher, features that also correlate with higher α -helix content (24, 25). Consequently, deconvolution gives similar helix contents of approximately 20–25%. Similarly, the β -sheet contents were both near 40%. Together, the CD spectra are consistent with the FT-IR data, indicating that the 45-kDa fragment has a similar overall secondary structure composition relative to the larger 63-kDa enzyme.

Theoretical locations of the secondary structural elements. Figure 7 shows the theoretically predicted locations of the β -sheet, α -helix, turn, and unordered structural elements by the algorithm of Garnier and colleagues (19). FT-IR and CD-based secondary structural data of full-length CBS was used to constrain the

Garnier predictions. This was done by choosing Garnier decision constants for each individual secondary structural component of CBS which corresponds to the average of decision constants derived previously for other proteins containing similar amounts of that particular structure (19). Under the constraint of the chosen decision constants, the Garnier method predicted a composition of 50.0% β -sheet, 28.8% α -helix, 9.9% turn, and 11.0% unordered structures from human CBS amino acid sequence (5). Twenty-two strands of β -extended structure, containing more than 5 amino residues, and 11 strands of α -helix, containing more

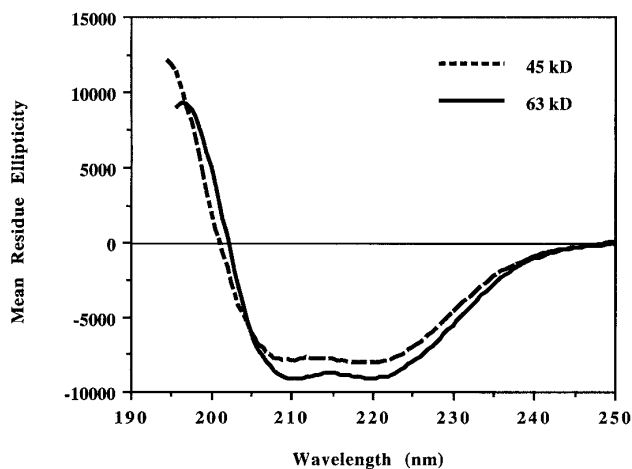


FIG. 5. Far UV-CD spectra of the truncated 45-kDa form and full-length 63-kDa CBS in 10 mM Tris-HCl buffer, pH 8.6, containing 150 mM sodium chloride. Solid line, truncated 45 kDa enzyme; broken line, full-length 63-kDa enzyme.

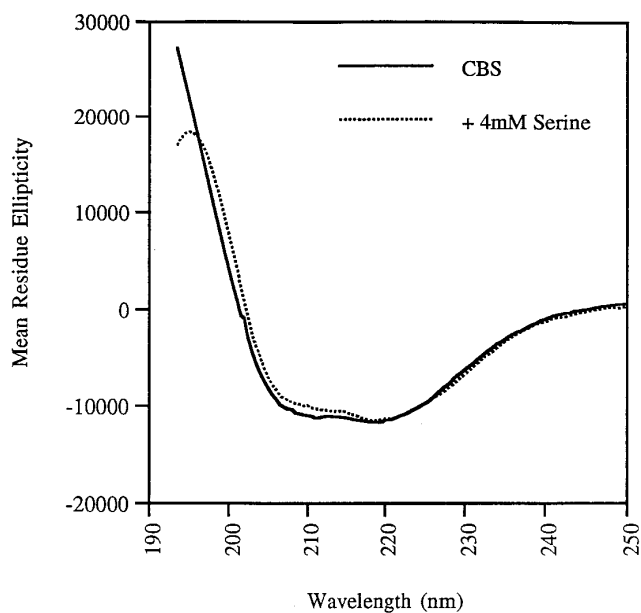


FIG. 6. Far UV-CD spectra of human CBS in the presence and absence of 4 mM serine in 10 mM Tris-HCl buffer, pH 8.6, containing 150 mM sodium chloride. Solid line, without serine; broken line, with serine.

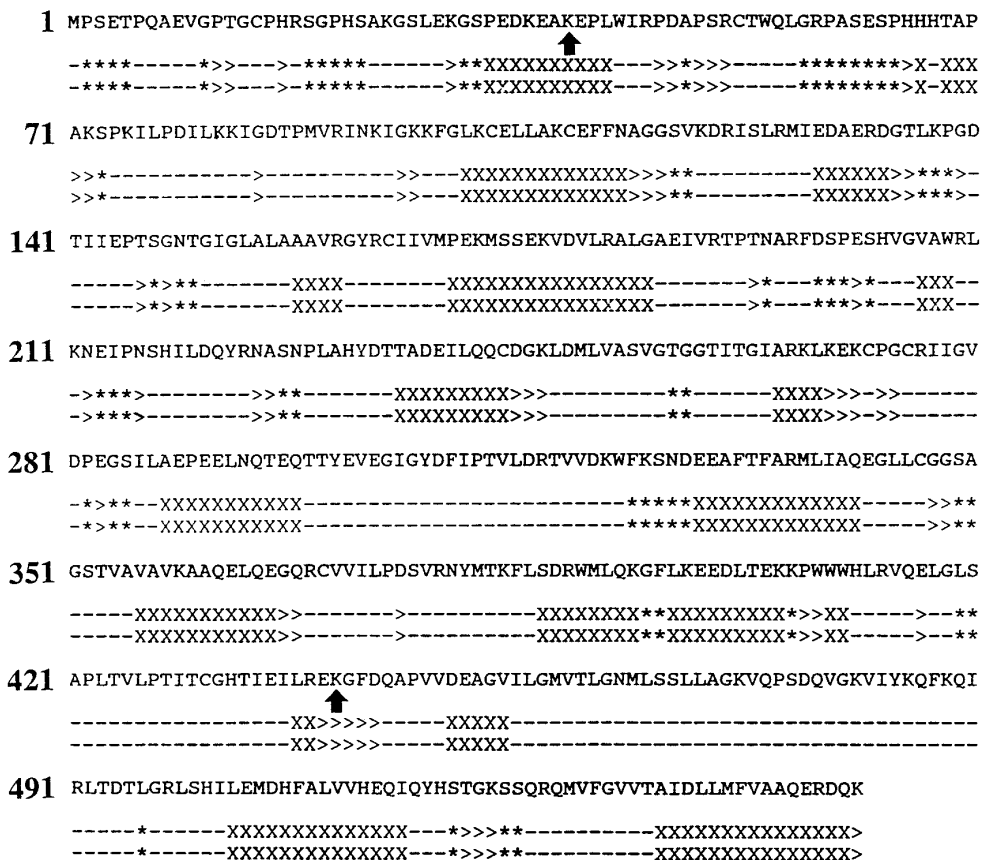


FIG. 7. Secondary structure of human CBS predicted by the Garnier's method as constrained by the FT-IR and CD-based secondary structural compositions. Under the constraint of the chosen decision constants, 50.0% β -sheet, 28.8% α -helix, 9.9% turn, and 11.0% unordered structures were predicted from human cystathionine β -synthase amino acid sequence. XXX, α -helix; ---, β -sheet; >>>, turn; ***, unordered. The positions of truncation are indicated by arrows.

than 8 amino acid residues, were predicted. The alternative distribution of β -extended strands and α -helices suggest that CBS may contain a β -sheet/ α -helix/ β -sheet structural domain.

DISCUSSION

Amide I band assignments of CBS. It is a well-known fact that the infrared amide I band frequency of a protein generally shifts to a lower wave-number as the protein is transferred from a H₂O medium to a D₂O medium, due to the isotopic effect of hydrogen to deuterium exchange at the N-H groups of the protein backbone (17, 20, 22). Assignments of amide I band frequencies to protein secondary structural elements have been established empirically for proteins in H₂O media (12) as well as in D₂O media (17, 20). However, the interpretation of infrared data of an individual protein in D₂O medium may not be straightforward. This is because of a strong dependence of deuterium-induced amide I band red-shift on the extent of H-D exchange at the protein backbone and on the nature of secondary structures. This dependence is especially strong for the

band components arising from highly ordered structures such as α -helix and β -sheet. Generally, the bands ascribed to α -helix are located at $1656 \pm 2 \text{ cm}^{-1}$ in H₂O media and red-shift to lower wave-number near 1650 cm^{-1} in D₂O media. The band component ascribed to high-wave-number β -sheet components are located at $1694 \pm 5 \text{ cm}^{-1}$ in H₂O media and red-shift to $1675 \pm 5 \text{ cm}^{-1}$ in D₂O media (12, 17). In practice, however, these general rules may not be applicable to a given protein. For example, the bands ascribed to the high-wave-number β -sheet component for concanavalin A (26), C-reactive protein, and serum amyloid P component (27) were not significantly affected by H-D exchange. The lack of isotopic effect on β -sheet-related band frequencies is likely due to low extent of deuterium exchange in β -sheet structures. Similarly, the bands near both 1656 and 1650 cm^{-1} can be assigned to α -helical structure in D₂O media (17, 20). A low extent of H-D exchange at α -helices of the protein can also affect the frequency shift of the band component ascribed to α -helix. As indicated by the coappearance of amide II and II' bands (Fig. 1), deuteration of the

full-length CBS is apparently incomplete even after 48 h dialysis against D₂O buffer at room temperature. Therefore, we tentatively assign the band at 1689 cm⁻¹ to high-wave-number β -sheet component and the band at 1651 cm⁻¹, in part, to α -helical structure.

Although the use of D₂O as a protein solvent may complicate infrared amide I band assignments, D₂O is a good alternative solvent especially when the solubility of a given protein is below 15 mg/mL, as is the case of CBS. Due to the low absorbance of D₂O in amide I region (12), a large path length (50 μ m) IR cell can be used to produce a high signal-to-noise ratio spectrum with a relatively low concentration of protein. We should point out that procedures involving repeated lyophilization of protein in D₂O media (28) or deuteration at higher temperature (29) have been used to overcome the problem of incomplete protein deuteration. Such a procedure was not used in our study to avoid possible protein denaturation. It is known that lyophilization could result in an irreversible denaturation of labile proteins (30, 31).

Substrate-induced conformational change. Protein conformational changes induced by binding of substrate or ligand are common for many proteins (12, 32). In the case of CBS, its overall secondary structural composition was not altered significantly by the binding of serine. This is evidenced by the lack of differences in far-UV CD spectra of CBS with or without 4 mM serine as well as the quantitative analysis of FT-IR spectra (Table I).

However, comparison of the second-derivative infrared spectra of the full-length CBS with or without serine has revealed significant changes at regions ascribed to α -helix and β -sheet structures (Figs. 2 and 4). These changes resulted mainly from the absorbance redistribution between bands assigned to same group of secondary structure: α -helix (1658 \leftrightarrow 1651 cm⁻¹) and β -sheet (1638 \leftrightarrow 1627 cm⁻¹) (Table II). We interpret these spectral changes as a substrate-induced conformational change involving a spatial rearrangement of secondary structural elements without a change in overall secondary structural composition. One of the advantages of the infrared spectroscopy in the conformational study of proteins is that it has greater capacity to detect such structural rearrangements than by CD spectroscopy. A total of 14 band frequencies can be identified and assigned to various secondary structural components in the amide I region of IR spectra of proteins (13), while only 4 major spectral patterns can be identified in the far-UV CD spectra of proteins (24, 25).

Truncated human CBS. The truncated CBS was first isolated and characterized by Kraus and colleagues (8). The enzyme was cleaved in the liver extract by a trypsin-like protease to the size of 48 kDa, which dimerized to 94 kDa in the native form. Similar to our 45-kDa truncated product, the UV-VIS spectrum of

the truncated 48-kDa enzyme resembled those of the 63-kDa parent and it was active (8). Our FT-IR and CD spectroscopic analysis supports the suggestion that the core structure of the truncated form retains all main features of the parent enzyme.

Secondary structure of CBS. Quantitative analysis of both FT-IR and CD spectra has revealed that human CBS contains 48–53% β -sheet, 40% α -helix/unordered, and 7–13% turn. The content of α -helical structure cannot be determined accurately from FT-IR spectra as a result of incomplete H–D exchange. However, under the constraint of the chosen decision constants, a theoretical prediction using the algorithm of Garnier and colleagues (19) gives a 29% α -helix, which is similar to the value of 25% of α -helix estimated from the CD spectra. Quantitative analysis by the infrared second-derivative method gives an accurate percentage estimate (\pm 5%) of secondary structural compositions of the proteins (12). For example, Caughey and colleagues reported in 1993 that bovine cytochrome c oxidase contains about 61% α -helix and 13% β -sheet structures (33), and later the X-ray crystallographic analysis showed that it contains about 58% α -helix and 9% β -sheet (34, 35). Dong and colleagues reported in 1994 that calcium-bound human C-reactive protein contains about 9% α -helix and 45% β -sheet structures (27), and later the X-ray crystallographic analysis showed that it contains about 5% α -helix and 42% β -sheet structures (36).

There are two noteworthy spectral characteristics for the CBS. First, only a small amount of turn structure (7–13%) was estimated from FT-IR and CD spectra. This value is much less than the average value (32%) for turn structure found in 29 other globular proteins (37). Second, the band assigned to high-wave-number β -sheet component accounts for only about 1% of the total amide I band area, while 48–53% of the total area is assignable to β -sheet structure. It has been known, theoretically and practically, that the high-wave-number β -sheet component associates specifically with the antiparallel β -sheet structure (12, 21) and the area ratio of high/low wave-number β -sheet components is 10–20% for proteins with an antiparallel folding (13, Protein Infrared Database⁴) and similar amount of β -sheet structure as the full-length CBS. Putting these two characteristics in perspective, we speculate that CBS contains predominantly parallel β -sheet and a small amount of antiparallel β -sheet. The α -helices, as well as turns, may serve as structures to connect the parallel β -sheet structures. The theoretically predicted locations for these secondary structures seems to agree with this view. As shown in Fig. 7, the full-length CBS may contain 11 strands of α -helices with more than 8

⁴ Part of the Protein Infrared Database can be obtained at the web site: <http://www.univnorthco.edu/chemist/aichun/irdata.htm>.

amino acid residues that serve to connect β -extended strands. Such a structural arrangement could be due to a central β -barrel structure with a β -sheet/ α -helix/ β -sheet conformation.

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