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Somatostatin conformation: Evidence for a stable intramolecular structure from circular dichroism, diffusion, and sedimentation equilibrium*

(tetradecapeptide/hypothalamic factor/proposed model)

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ABSTRACT Somatostatin is a hypothalamic tetradecapeptide that inhibits the release of growth hormone, insulin, and glucagon. The circular dichroism spectrum is characterized by negative extrema at 238 nm and 270 nm, and a positive extremum at 225 nm. The far ultraviolet circular dichroism spectrum is consistent with the presence of ordered secondary structure such as β-structure, but not α-helix. Sedimentation equilibrium results demonstrate that somatostatin exists in its monomeric form (i.e., a molecular weight of 1610 ± 36 was obtained) and, thus, the structure must arise from intramolecular interactions. The diffusion constant of somatostatin was estimated to be 1.66×10^{-6} cm²/sec. These data are consistent with an ellipsoidal rather than a spherical shape. The magnitude of the ellipticity at both 225 nm and 238 nm is quite dependent on guanidinium hydrochloride concentration; the midpoint occurs at about 3 M and the transition is cooperative-like. These data strongly suggest that somatostatin has a stable conformation in aqueous solution. A model, consistent with the results of the physicochemical studies and with semi-empirical rules for secondary structure formation, is proposed for somatostatin. The proposed structure consists of a hairpin loop with several residues in an antiparallel β-pleated sheet, is somewhat elongated, and contains a hydrophobic domain at one end and a hydrophilic domain at the other end.

Somatostatin is a hypothalamic (cyclic) tetradecapeptide which has been purified, sequenced, and synthesized (1–4). The sequence of the reduced form is as follows: NH_2-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-COOH. In addition to inhibiting growth hormone release from the anterior pituitary (1), somatostatin also inhibits the release of both glucagon and insulin from the pancreas (5). These observations have important implications for potential therapeutic uses of somatostatin, e.g., in diabetes (6); moreover, it is anticipated that somatostatin will prove quite valuable as a tool in elucidating complex metabolic interrelationships such as glucose homeostasis.

In addition to their extensive work on the purification and characterization of somatostatin, Guillemin, Rivier, and coworkers have prepared a large number of synthetic derivatives and assayed these for biological activity (4, 7–9). However, no studies have appeared on the conformational aspects of somatostatin. Our laboratory has been engaged in a number of studies aimed at elucidating more quantitatively the contributions of the aromatic and disulfide chromophores to the circular dichroism spectra of proteins, hormones, and fragments (10–15). Similar studies on somatostatin, which are described herein, have led us to conclude that this tetradecapeptide exhibits a stable intramolecular structure in aqueous solution and a tentative conformation is suggested.

Abbreviations: CD, circular dichroism; GdmCl, guanidinium hydrochloride.

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MATERIALS AND METHODS

Somatostatin (cyclic) was purchased from Pierce Chemical Co. (lot no. 5164-8), Gly-Phe-Gly (lot G1704) from Cyclo Chemical Co., and spectrophotometric grade guanidinium hydrochloride (GdmCl) from Heico, Inc.

Amino-acid analyses were done on 100–300 μg amounts of reduced (dithiothreitol in 6 M GdmCl), S-carboxymethylated (iodoacetate) somatostatin hydrolyzed in 0.6 ml of 4 M methanesulfonic acid containing 0.2% of 3-(2-aminoethyl)-indole for 22 hr at 115° (16). Hydrolysates were analyzed on a Beckman 120C amino-acid analyzer in the usual manner. Sulfhydryl groups were analyzed using the sensitive method described by Rohrback *et al.* (17).

Circular dichroism (CD) spectra were obtained on a Cary 60 spectropolarimeter with a model 6002 CD attachment (calibrated with *d*-10-camphorsulfonic acid) using pathlengths of 0.05, 0.5, 1, and 2 cm. The cell was maintained at a constant set temperature using a thermostatted cell block and a circulating water bath. Experimental protocol and data reduction were as described elsewhere (11, 13, 15). Unless indicated otherwise, $[\theta]$ represents the mean residue ellipticity in degree·cm²/decimole and rotational strengths, R, are in cgs units.

Sedimentation equilibrium data were obtained using a model E ultracentrifuge equipped with electronic speed control and photoelectric scanner. Sedimentation equilibrium was carried out using the conventional low-speed method of Van Holde and Baldwin (18) with a 12 mm centerpiece and a solution column height of 2.8 mm. Somatostatin (0.11 mg/ml), dissolved in 0.1 M KCl, 10 mM Tris·HCl at pH 7.0, was analyzed after 12 hr at 30,000 rpm, 20°.

The diffusion constant was determined by following boundary spreading in a synthetic boundary cell using the photoelectric scanner and plotting Δr^2 versus t, where Δr is the distance from $c/4$ to $3c/4$ (19). Somatostatin (0.07–0.22 mg/ml) was in a similar buffer to that used in sedimentation equilibrium. An AN-J rotor was used to minimize convection arising from drive vibrations and the rotor and cell were allowed to reach complete thermal equilibrium at 20° prior to acceleration to 8000 rpm to form the boundary.

RESULTS

The amino-acid composition of S-carboxymethylated somatostatin was in excellent agreement (±5%) with that expected from the sequence (1) with the single exception that tryptophan appeared somewhat low (e.g., 0.5–0.85 mol/mol of somatostatin). Using similar conditions with other proteins

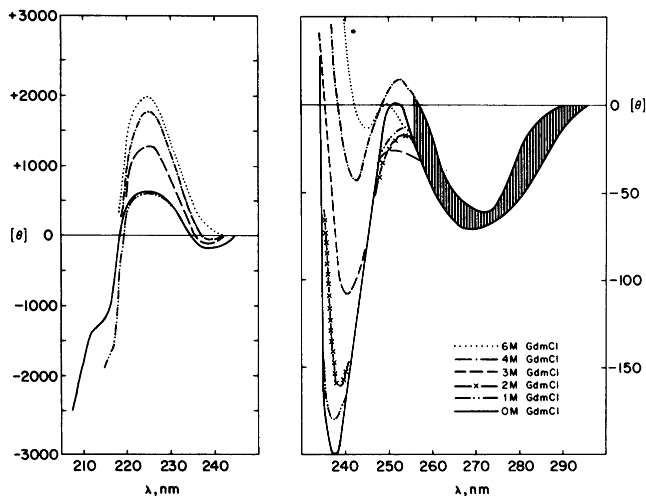

FIG. 1. CD spectra (mean-residue ellipticity versus wavelength) of somatostatin at several GdmCl concentrations. The 0 M solvents contained 0.1 M KCl, 10 mM Tris-HCl at pH 7. All spectra were recorded at 25°.

(i.e., hydrolysis of small amounts), we have frequently observed nonquantitative recovery of tryptophan. Thus, the apparent low tryptophan content is attributed to the hydrolysis method and not to the sample. The results of others on somatostatin also support this interpretation. Using hydrolysis conditions similar to those reported here, 0.5 mol of tryptophan were found per mol of somatostatin; in contrast, enzymic hydrolysis yielded 1.1 mol of tryptophan (3, 4). Somatostatin was analyzed for the presence of free sulfhydryl groups and none were detected. Under the conditions used and the sensitivity of the method, we can conclude that over 99% of the molecules are in the cyclic form. (The possibility of intermolecular disulfides is ruled out by hydrodynamic results to be presented below.)

The CD spectrum of somatostatin at various GdmCl concentrations is shown in Fig. 1. The shaded area from 255 to 300 nm represents the variation obtained from 0 to 6 M GdmCl and the small differences found in this area of the CD spectrum are well within the standard error. However, major changes are seen near 240 nm and 225 nm as a function of solvent change. The far-UV CD spectrum in buffer is quite striking in that somatostatin contains four aromatic chromophores and a single disulfide which have an expected contribution together well in excess of +3000 deg·cm^2/dmol (15, 20), while the observed CD magnitude at 220–230 nm is very small.

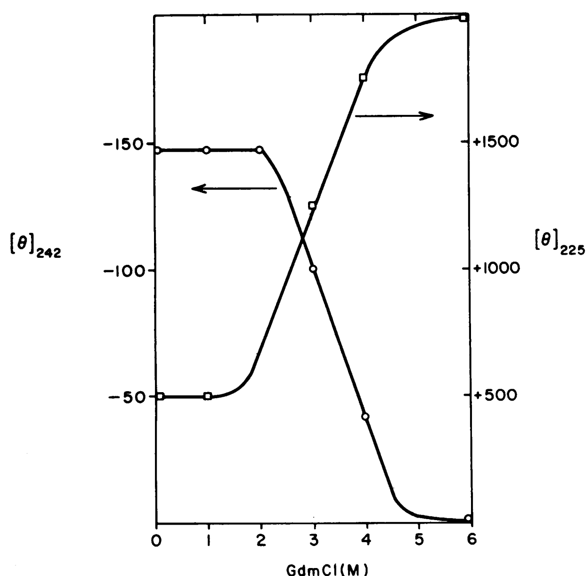

FIG. 2. The mean residue ellipticity at 225 nm and 242 nm of somatostatin in several GdmCl concentrations at 25°.

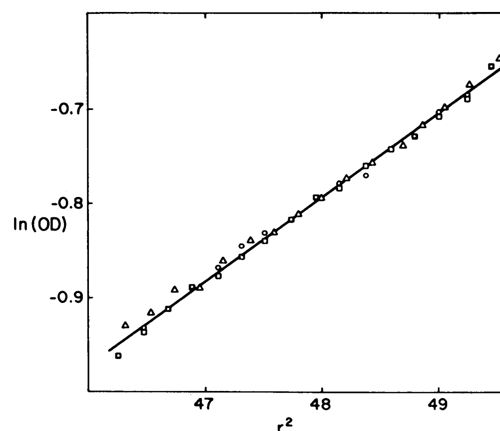

FIG. 3. Sedimentation equilibrium analysis of somatostatin at 20°; the plot shows ln(OD) versus r^2 after 12 hr at 30,000 rpm. The least-squares line corresponds to a molecular weight of 1610 ± 36 and the symbols represent three separate scans.

As shown in Fig. 2, the changes in $[\theta]$ at 242 nm and 225 nm are very nearly coincident as the denaturant concentration is increased to 6 M. Moreover, the major portion of the spectral changes occurs between 2 M and 4 M GdmCl and has the appearance of a cooperative-like transition (11). Based on an analogy with other proteins (21), the conformation of somatostatin should be more disordered in concentrated GdmCl.

One possible explanation for these changes could be that somatostatin exists in solution as an aggregate, and the changes observed in the CD spectrum may result solely from a dissociating effect of GdmCl. In order to examine this possibility, we carried out a sedimentation equilibrium experiment on somatostatin and Fig. 3 illustrates the data obtained after 12 hr at 30,000 rpm. The partial specific volume, $\bar{v}$, was estimated (22) to be 0.723 using the known amino-acid composition (1). The least squares line through the points yields a molecular weight of 1610 ± 36; the value calculated from the amino-acid sequence is 1636. The ln(OD) versus r^2 points were also fitted to a parabola in r^2. The coefficient of the square term, $(r^2)^2$, was not significantly different from zero at a confidence level of 95%, indicating that somatostatin exists predominantly in the monomeric form under these conditions.

In the diffusion studies, plots of Δr^2 versus t were linear and yielded an estimate for $D°_{20,w}$ of $(1.66 \pm 0.3) \times 10^{-6}$ cm^2/sec. Assuming 1 g of water bound per g of somatostatin, the hydrodynamic data are consistent with a prolate ellipsoid of axial ratio 5. In view of the inherent assumptions involved in the estimation of axial ratios of small solute molecules, this result should be considered tentative.

The temperature dependence of the 225 nm CD extremum is shown in Fig. 4. In 6 M GdmCl, where there should be little if any ordered structure, the ellipticity decreases as the temperature is raised. This type of thermal dependence qualitatively resembles that observed for the nonordered polypeptide corticotropin, the tripeptide Ala-Ala-Ala, and aromatic containing oligopeptides in 6 M GdmCl (15). At a concentration of 3 M GdmCl, where somatostatin contains appreciable structure (see Fig. 2), $[\theta]_{225}$ increases with in-

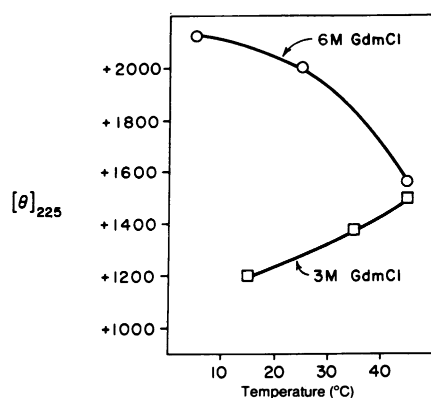

FIG. 4. Plot of $[\theta]_{225}$ versus temperature for somatostatin in 3 M and in 6 M GdmCl.

creasing temperature to a value at 45° which is about the same magnitude as observed in 6 M GdmCl. Presumably, this increase of $[\theta]_{225}$ in 3 M GdmCl arises from a thermal melting of ordered regions. It would appear, then, that the tetradecapeptide is in a more disordered state in 6 M GdmCl and, that in 3 M GdmCl, a similar conformational state may be achieved by increasing the temperature to 45°. At a concentration of 1 M GdmCl no appreciable increase in $[\theta]_{225}$ is seen on increasing the temperature to 45°, thus indicating thermal stability under these conditions.

Table 1 gives the rotational strengths of the 225 nm and 238 nm resolved CD bands as a function of GdmCl concentration. It should be noted that the 238 nm band does not completely vanish in 6 M GdmCl. In order to complete the model compound data previously reported (11), the resolved gaussian band parameters for Gly-Phe-Gly in 6 M GdmCl are given in Table 2. As shown earlier, a large negative CD band appears at 238 nm for Gly-Phe-Gly in 80% ethylene glycol, but is not present in water (11). A similar observation on another model compound has also been made (23). As Table 2 shows, the phenylalanyl chromophore in 6 M GdmCl also lacks this large negative band.

DISCUSSION

The spectroscopic data lead to the conclusion that somatostatin has a stable conformation in aqueous solution and the hydrodynamic data suggest that the structure is elongated and arises from intramolecular interactions. It seems likely that the CD spectral changes observed with increasing GdmCl concentration result from a more or less cooperative unfolding of some type of ordered structure(s). The midpoint of the unfolding profile occurs at about 3 M GdmCl, indicating a reasonable degree of stability of the native structure.

Table 1. Rotational strengths* of the major GdmCl-dependent CD bands of somatostatin

GdmCl, M	λ_0, nm	$R \times 10^{41}$, cgs units	λ_0, nm	$R \times 10^{41}$, cgs units
0	225	2.50	236	−1.08
1	225	2.50	236	−1.01
3	225	4.80	238	−0.61
4	225	7.20	238	−0.27
6	225	8.78	238	−0.12

* The rotational strength was determined from the parameters of the resolved gaussian bands according to the following equation:

$$R \simeq 1.234[\theta°](\Delta/\lambda_0) \times 10^{-42}$$

where $[\theta°]$ is the extremum (mean residue) ellipticity of the resolved band at wavelength λ_0, and Δ is the half-bandwidth (20).

Table 2. Resolved gaussian band parameters* for glycyl-L-phenylalanyl-glycine† in 6 M GdmCl

Assignment	λ_0, nm	$[\theta°]$, degree·cm²/decimole	$R \times 10^{42}$, cgs units
0–0	267.5	−144	−1.34
0+520	263.5	+45	+0.42
0+930	261.2	−150	−1.41
0+520+930	257.5	+31	+0.30
0+2(930)	255	−81	−0.67
0+520+2(930)	252.5	+10	+0.15
‡	238	+100	+2.59

* $[\theta°]$ and R refer to the molar ellipticity and molar rotational strength based on the phenylalanine moiety, respectively.

† The amino-acid composition of the tripeptide (hydrolyzed in 6 M HCl, 110°, 20 hr) gave Gly:Phe molar ratios of 2.05:1.00 and no other detectable amino acids. The tripeptide migrated as a single component using thin-layer chromatography and three different solvent systems.

‡ No assignment has been made to this band.

It is of interest to identify the class of chromophore responsible for the ellipticity in the vicinity of 236–238 nm since the magnitude of this band is very sensitive to GdmCl concentration. It does not appear to result from the disulfide chromophore since it is also present in S-carboxymethylated somatostatin (24). In recent model compound work, we have found that the tryptophanyl chromophore exhibits no bands near 238 nm in water, 80% ethylene glycol, or 6 M GdmCl (15). However, as discussed in the previous section, the phenylalanyl chromophore is characterized by a marked negative band at 238 nm when dissolved in 80% ethylene glycol, but not in water or 6 M GdmCl. Thus, the CD data are compatible with a model for native somatostatin in which one or more of the phenylalanyl residues are partially shielded from the solvent forming a hydrophobic area. At high GdmCl concentrations, the molecule unfolds into a more disordered state, the masked phenylalanyl side chains gain rotational freedom and are more completely exposed to the solvent. Consequently, the 238 nm negative CD band is diminished.

The variation in the 220–225 nm positive CD band is also of interest in considering a model for somatostatin structure. As noted above, from model compound data we expect a positive band at 220 nm with a magnitude between about 3000 and 4000 deg·cm²/dmol. As the GdmCl concentration increases to 6 M, $[\theta]_{225}$ approaches +2000. For reduced, carboxymethylated somatostatin in 6 M GdmCl, the value of $[\theta]$ closely approaches that expected from the model compound data (24). The variation in $[\theta]_{225}$ with GdmCl concentration may result both from changes in the orientation of the aromatic chromophores and alterations in the arrangement of the peptide ϕ,ψ angles with denaturation. The second possibility is favored by the observation that the near-UV CD spectrum of somatostatin above 250 nm is not particularly sensitive to GdmCl concentration. Thus, it seems reasonable that for native somatostatin there is a peptide contribution at 225 nm of approximately −2000 to −4000 deg·cm²/dmol and this is reduced to about 0 to −2000 deg·cm²/dmol in 6 M GdmCl. A peptide contribution of −3000 deg·cm²/dmol for native somatostatin at 220–225 nm would be compatible with the existence of some four to six residues in an antiparallel β-sheet (25, 26).

A space-filling model of somatostatin, consistent with the

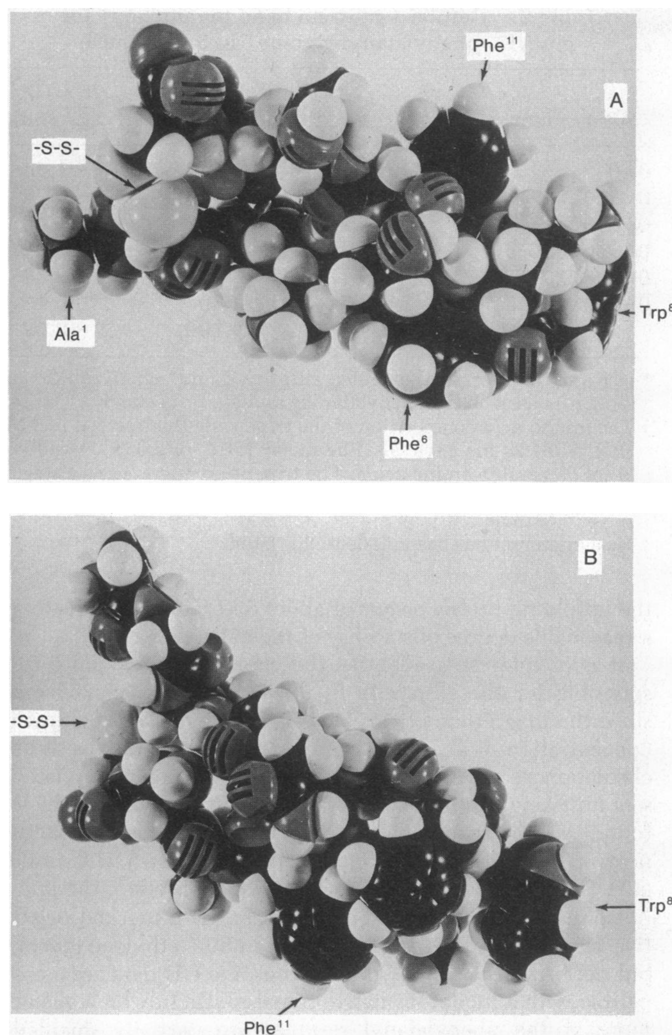

FIG. 5. A space-filling model of the proposed conformation for somatostatin. (A) NH_2-terminal Ala and the disulfide bond appear at the left; Phe in position 6 and 11 appear in the lower and upper right-hand portions, respectively. Trp in position 8 is at the far right. (B) This view shows the hydrophobic area created by the aromatic residues folding over the β-sheet area.

above physicochemical results, has been constructed and is shown in Fig. 5. The proposed structure is somewhat elongated and forms a hairpin loop with four residues in an antiparallel β-pleated sheet. Three peptide-peptide hydrogen bonds are formed and these are partially shielded from solvent by the phenylalanines and the tryptophan. Interestingly, application of the method of Chou and Fasman (27) to somatostatin leads to the prediction that residues 6 to 12 would be in a β-pleated sheet with $\langle P_\beta \rangle = 1.16$ and $\langle P_\alpha \rangle = 1.03$. (The computer program for this analysis was kindly provided by Mr. R. Glenn Hammonds, Jr.) The proposed conformation is characterized by a hydrophobic domain on one end of the structure and a hydrophilic domain containing the disulfide on the other end.

The model is tentative and additional studies, such as high-resolution nuclear magnetic resonance or preferably x-ray crystallography, are essential for validation. In any case, the proposed structure should prove useful as a guide to the preparation of synthetic analogs (9).

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