CONFORMATION OF SOMATOSTATIN USING SCALAR COUPLING CONSTANTS FROM 270 AND 600 MHz SIMULATED PROTON MAGNETIC RESONANCE SPECTRA

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ABSTRACT The conformation of the 14 amino acid peptide hormone somatostatin in aqueous solution was investigated through a proton magnetic resonance (PMR) scalar coupling analysis. Experiments were performed at two fields, 270 and 600 MHz, and included double and triple resonance difference scalar decoupling, resolution enhancement and computer simulation. The agreement between simulated and observed spectra at both fields provided support for the correctness of the analysis. The resultant scalar coupling constants, $^3J_\alpha$H–NH and $^3J_\alpha$B, gave information on the backbone (ϕ) and side chain (χ^1) torsional angles, respectively, which eliminated either of the proposed conformations of somatostatin as describing a predominant conformer of the molecule in solution under our conditions.

INTRODUCTION

Somatostatin is a peptide hormone with the following primary structure:

$$\underset{\text{Ala}^1\text{Gly}^2\text{Cys}^3\text{Lys}^4\text{Asn}^5\text{Phe}^6\text{Phe}^7\text{Trp}^8\text{Lys}^9\text{Thr}^{10}\text{Phe}^{11}\text{Thr}^{12}\text{Ser}^{13}\text{Cys}^{14}}{\text{Ala}^1\text{Gly}^2\text{Cys}^3\text{Lys}^4\text{Asn}^5\text{Phe}^6\text{Phe}^7\text{Trp}^8\text{Lys}^9\text{Thr}^{10}\text{Phe}^{11}\text{Thr}^{12}\text{Ser}^{13}\text{Cys}^{14}}.$$

Preliminary reports of the assignments and analysis of the proton NMR spectrum of somatostatin (1–3)[1] and the data used to assess proposed somatostatin conformations have been given[2] (4, 5).

Here we report (*a*) our detailed difference scalar decoupled (DSD) experiments at 270 and 600 MHz, which yielded analyses of almost all aliphatic proton spin systems of somatostatin, (*b*) proof of the correctness of the PMR parameters by comparison of the simulated and experimental spectra at two fields, (*c*) a conformational analysis of the side chains of somatostatin, and (*d*) a discussion of proposals for the secondary conformation based upon our backbone scalar coupling constants ($^3J_\alpha$H–NH), temperature dependencies of amide chemical shifts ($\Delta\delta/\Delta T$), and hydrogen-to-deuterium exchange studies.

EXPERIMENTAL PROCEDURE

The details of the 270 MHz experiments were given in the preceding papers (1, 2). In the triple resonance experiments at 270 MHz the decoupler pulse was frequency modulated with a Hewlett-Packard 3300A function generator (Hewlett-Packard Co., Palo Alto, CA).

The 600 MHz spectra were obtained in the correlation mode at the NMR facility for Biomedical Studies in the Carnegie-Mellon Institute. Amide spectra were obtained with a steady-state water-eliminated Fourier transform pulse sequence at 270 MHz and in the correlation mode at 600 MHz (6).

Simulations were performed on a Nicolet 1180 computer (Nicolet Instrument Corp., Madison, WI) using the ITRCAL program or NTCSIM with the NTCFT-1180 program (Nicolet).

RESULTS AND DISCUSSION

Experiments in H_2O

Amide Parameters. The amide chemical shifts and $^3J_\alpha$H–NH values were taken from the frequencies of the observed amide resonances at either 270 or 600 MHz. The amide assignments, Table I, were made by decoupling at the α proton positions and observing doublet-to-singlet collapse of the amide resonances.

The temperature dependencies of the amide chemical shifts (Table I) indicated that the amides of three residues, Trp^8, Lys^9, and Thr^{10}, were exposed to the solvent to a lesser degree than were other amides (2). Consistent with this result was the finding that upon dissolution of the fully protonated peptide in D_2O the amides of these residues had the slowest H-to-D exchange rates, Table I. Additional

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[1]Reference 3 confirmed most of the assignments and *J* values reported in reference 1, and *b* of footnote 2, corrected our Cys^{14} and Phe^7 assignments, but did not separately assign Phe^6 and Phe^7.

[2]Preliminary findings on the scalar coupling analysis and conformational analysis of the proton NMR spectrum were reported by us at (*a*) The Pittsburgh NMR Conference, December, 1979, and (*b*) The Gordon Conference on Peptides (February, 1980) and on NMR Spectroscopy (August, 1980). Reference 1 contained a summary of the assignments. The assignment process using synthetic analogs was fully detailed in reference 2.

TABLE I
AMIDE PARAMETERS*

	δ, PPM			J, Hz‡		$\frac{\Delta\delta \text{ ppb}}{\Delta T, \text{ deg}}$§	k‖	$\frac{k_{obs}}{k_{pred}}$¶
	270**	600‡‡	Tetra§§	270**	600‡‡			
Gly^2	8.64	8.65	8.39	$11._2$‖‖	$13._0$‖‖	−5.5	>1	>.2
Cys^3/Phe^{11}¶¶	8.48	8.48	8.31 (Cys)	$7._0$	$7._5$	−7.6	1	4 (10) (Cys) (Phe)
	(8.20)	8.21	8.23 (Phe)	$(7._2)$	$8._6$	(−6.1)	(0.3)	
Lys^4	8.57	8.61	8.41	$7._0$	$7._1$	−8.3	0.8	8
Asn^5	(8.18)	8.18	8.75	$(8._0)$	$11._2$	(−6.1)	(0.30)	
Phe^6/Thr^{12}***	(8.05)	(8.06)	8.23 (Phe)	$(4._8)$	$(6._3)$	(−7.0)	(0.18)	
	(8.04)	(8.04)	8.24 (Thr)	$(8._8)$	$(8._6)$	(−7.0)	(0.20)	
Phe^7	(8.07)	(8.08)	8.23	$(8._0)$	$(7._8)$	(−7.0)	(0.25)	
Trp^8	7.67	7.67	8.10	$7._0$	$6._7$	−4.6	$.06_7$	0.8_1
Lys^9	7.85	7.86	8.41	$7._2$	$6._0$	−3.1	0.17	1.9
Thr^{10}	7.79	7.80	8.24	$8._0$	$9._0$	−4.3	0.1_3	0.5_7
Ser^{13}	8.12	8.12	8.38	$8._0$	$8._2$	−5.9	(0.20)	
Cys^{14}	8.28	‡‡‡	8.31	$8._2$	‡‡‡	−6.7	>1	

*All amide protons except one were assigned by 270 MHz underwater decoupling in the α region. Decoupling at the position of the overlapping Phe^6 and Thr^{12} α protons resulted in collapse at only one position, ~8.05 ppm. The peak intensities and number of lines at ~8.05 ppm indicated that two protons occur at this position. Accurate determination of some values was prevented by amide proton overlap; these have been estimated and are shown within parentheses.

‡J = observed peak splitting × 1.09 (electronegativity correction).

§Measured over the interval 298°–318°K, 270 MHz, pH 4.3.

‖Approximate values in 10^{-3} s^{-1} for the observed rate of exchange at pD = 2.8 and 12°C. The values for the pseudo–first-order rate constants, k, were obtained from least-squares fits of data to $\ln I/I_0 = -k\,t$ where I was the amide's peak height, I_0 was the most downfield Trp aromatic proton's peak height in the same spectrum, and t was the time elapsed from the addition of D_2O to the peptide in the protonated form. Standard deviations in the slopes were 7–25% of the slope values. Estimated values are given in parentheses for six amides where overlap of resonances contributed significantly to error in measurement. As can be seen from the chemical shifts and from Figs. 1–3 in reference 2, these six amide resonances occurred within a 0.16 ppm range; overlap was much less in the two other groups of three resonances for which the ranges were 0.16 and 0.18 ppm.

¶The k_{obs} values are those given in the adjacent column. The k_{pred} values were determined using the method of Englander et al. (14, 15), $k_{pred} = k_{base} + k_{acid} + k_{H_2O}$. At 20°C the k values (s^{-1}) were as follows: $k_{base} = 3.57 \times 10^{pD-7} \times C_{BL} \times C_{BR}$, $k_{acid} = 0.275 \times 10^{-pD} \times C_{AL} \times C_{AR}$, $k_{H_2O} = 4.4 \times 10^{-4}$. The values for k_{base}, k_{acid} and k_{H_2O} at 12°C were calculated from the 20°C values using activation energies given in Englander et al. (14), 17.4, 13, and 28 kcal, respectively. C_{BL} and C_{AL} were corrections that depend on the identity of the amino acid residue of the amide in question ($C_{BL} = C_{AL} = 1$ for alkane side chains, the values of C_{BL} and C_{AL} for other amino acid residues were given in reference 15 as log C values). C_{BR} and C_{AR} were corrections similar to C_{BL} and C_{AL} except that they were determined by the identity of the amino acid residue preceding the amide in question. The correction factors used for Trp were those given for Phe and Tyr in reference 15.

**pH 4.3, 298°K.

‡‡pH ~3.8.

§§Random coil values from Bundi and Wuthrich (8) for the amino acid residue X in the tetrapeptide H-Gly-Gly-X-Ala-OH in H_2O at pH 2.2–5.0, 308°K.

‖‖Sum of $^3J_{\alpha H\text{-}NH}$ and $^3J_{\alpha' H\text{-}NH}$.

¶¶Overlap of the Cys^3 and Phe^{11} α protons prevented separate assignment of the amide protons at 8.48 and 8.20 ppm.

***Overlap of the Phe^6 and Thr^{12} α protons prevented assignment of the amide protons at 8.05 and 8.04 ppm.

‡‡‡At the pH of the 600 MHz experiments the Cys^{14} amide proton was downfield shifted, outside of the region scanned.

support for solvent shielding of these three amides came from the observation that the three amide resonances were observed in H_2O at higher pH values than were those of other somatostatin amides. The three $\Delta\delta/\Delta T$ values (−4.6, −3.1, −4.3, ppb deg^{-1}) were not as low, however, as those that have been observed under similar conditions for peptide amide protons serving as hydrogen bond donors in a predominant solution conformer (7).

Possible interpretation of these data are (*a*) some conformational feature existed that involved amide protons of residues 8, 9, and 10 in shielding from the solvent to a lesser degree than if they were in hydrogen bonds. It is interesting to note (Table I) that the deviation of somatostatin amide chemical shifts from the corresponding random coil values (8) were greatest for residues 5, 8, 9, and 10. (*b*) There was conformational averaging among conformers where these amides participated in hydrogen bonding. In either case the data do not support one of the suggested turn conformers (4, 5) as the single or predominant species in aqueous solution because in each of these conformers only one of the three amides of residues 8–10 is involved in an intramolecular or hydrogen bond (Table II).

TABLE II
PROPOSED β TURNS

Model	Parameter	Phe[7]	Trp[8]	Lys[9]	Thr[10]	Phe[11]
*A**	Position in Turn	1	2	3	4	
	Solvent Exposure‡	bond (11)	exp	exp	bond (7)	
	Type II′ ϕ§		+60	−80		
	$^3J_{pred}$‖		8.5	7.2		
	$^3J_{obs}$		7.0	7.2		
B¶	Position in Turn		1	2	3	4
	Solvent Exposure‡		bond (11)	exp	exp	bond (8)
	Type I** ϕ§			−60	−90	
	$^3J_{pred}$‖			3.5	8.8	
	Type I′** ϕ§			+60	+90	
	$^3J_{pred}$‖			8.5	6.8	
	Type II** ϕ§			−60	+80	
	$^3J_{pred}$‖			3.5	7.7	
	Type II′** ϕ§			+60	−80	
	$^3J_{pred}$‖			8.5	7.2	
	$^3J_{obs}$			7.2	8.0	

*Model A was suggested in reference 4 as the biologically active conformation of somatostatin at the receptor.
‡The NH of the given residue is classified as either exposed to the solvent (exp) or hydrogen bonded (bond) to the carbonyl oxygen of the residue whose number is given in parentheses.
§The backbone torsional angles for the various turns were taken from (16) and are given in degrees.
‖The predicted coupling constants ($^3J_{NH\text{-}CH}$), corresponding to the given values of ϕ, were obtained from Karplus type curves in reference 17 and are given in Hz.
¶Model B was suggested in reference 5.
**The type of turn for *B* was not specified in reference 5. In sequences of L-amino acids that do not contain Gly the type *I* turn has the least amount of unfavorable steric interactions. Less favored are types I′, II and II′. An analysis of the occurrence of β turns (16) found examples of types I, I′, and II but not of type II′ β turns in sequences that did not contain glycine.

The $^3J_{\alpha}$H–NH values corroborate this conclusion, since the observed values are not those predicted by either of these models (Table II). The data do not preclude the existence of some percentage of either or of both the conformations.

Determination of *J* and δ Values in D_2O

The method used to obtain $^3J_{\alpha\beta}$ values from spectroscopic data depended on the complexity of the spin system and the amount of overlap with other resonances. In the simplest cases the analysis of the spin system (determination of the pertinent α and side chain proton δ and *J* values) could be taken directly from the frequencies of the observed resonances at 270 MHz. This was true for Ala[1], Gly[2], Thr[10], and Thr[12]; in the case of the Thr β protons, only the approximate values of the chemical shifts were found in this way.

The frequencies of additional resonances were obtained from the resolution enhanced (9) 270 MHz spectra. The δ and *J* values for Asn[5] were obtained in this way.

Experiments at 600 MHz facilitated analysis in two ways. At the higher field the overlap between resonances of different amino acid residues was reduced, and strongly coupled spin systems became less strongly coupled as $\Delta\delta/J$ increased at the higher field, making it easier to obtain approximate δ and *J* values. Iterated computer simulations starting with these values yielded refined values that gave calculated spectra agreeing well with experimental spectra. The *J* and δ values for the spin systems of Phe[6] and Trp[8] were obtained in this way as were the chemical shifts of the Thr β protons. Frequencies of the Lys[9] α proton resonances at 600 MHz were used in its analysis.

The resonances of the α and β protons of four amino acid residues overlapped with each other (Cys[3], Phe[7], Phe[11], and Cys[14]). Approximate δ and *J* values for these residues were obtained from DSD spectra (10) at 270 MHz and the final values were obtained from iterated computer simulations.

Triple resonance experiments at 270 MHz and standard scalar decoupling experiments (double resonance) at 270 MHz and 600 MHz were used to analyze the Lys[9] β protons. The small difference in chemical shift between the two Lys[4] β protons and their strong coupling to the Lys[4] γ protons prevented a full scalar coupling analysis of this residue.

Simulated D_2O Spectrum of Somatostatin

The chemical shifts and coupling constants resulting from the analysis are contained in Table III. From these parameters the individual amino acid residue spectra were simulated and then summed to yield a simulated spectrum of almost the whole somatostatin molecule.

The sum of the individual residue spectra is compared with the experimental spectrum in Figs. 1–4. Figs. 1 and 2 contain the α and β regions at 270 MHz; Figs. 3 and 4 contain the same regions at 600 MHz. The good agreement between simulated and experimental spectra at the two fields supports the reasonableness of the analysis.

TABLE III
CHEMICL SHIFTS AND COUPLING CONSTANTS IN D_2O

Residue	Chemical shifts			Coupling constants			Other δ and J values
	α	β*		$^3J_{\alpha\beta}$*		$^2J_{\beta\beta}$	
	ppm	*ppm*		*Hz*		*Hz*	
Ala[1]	4.01	1.49		7.3			
Gly[2]	4.00						
Cys[3]	4.68	3.15	2.99	5.9	7.7	−14.3	
Lys[4]	4.23	~1.7	~1.7				$\delta_\gamma \simeq 1.35$, $\delta_\delta \simeq 1.58$
							$\delta_\epsilon = 2.93$, $J_{\delta\epsilon} = 7.7$
Asn[5]	4.56	2.62	2.59	5.8	8.0	−15.6	
Phe[6]	4.39	2.75	2.72	8.9	5.9	−14.1	
Phe[7]	4.47	3.01	2.97	6.6	8.0	−14.2	
Trp[8]	4.60	3.28	3.25	5.6	7.4	−15.3	
Lys[9]	4.14	1.70	1.59	5.4	9.3	−13.6	$\delta_\gamma \simeq 1.05$, $\delta_\delta \simeq 1.55$
							$\delta_\epsilon = 2.86$, $J_{\delta\epsilon} = 7.8$
Thr[10]	4.30	4.18		4.4			$\delta_\gamma = 1.11$, $J_{\beta\gamma} = 6.3$
Phe[11]	4.66	3.18	3.08	5.7	8.5	−14.4	
Thr[12]	4.4	4.18		4.4			$\delta_\gamma = 1.15$, $J_{\beta\gamma} = 6.3$
Ser[13]	4.53	3.89	3.87	5.8	5.1	−11.7	
Cys[14]	4.49	3.20	3.05	4.7	7.6	−14.1	

*In the ABX spin system residues the first value of the parameter (either δ_β or $J_{\alpha\beta}$) is for the downfield β proton and the second value is for the upfield β proton.

Side Chain Rotamers

Information on the peptide side chain torsional angles, χ^1, was obtained from the $^3J_{\alpha\beta}$ coupling constants. The population distributions of the side chains of the individual amino acid residues among the classical rotamers ($\chi^1 = 180°, 60°, -60°$) were calculated. It was assumed that the side chain orientations were confined to those of the classically allowed staggered rotamers, and that $^3J_{trans} = 13.59$ Hz and $^3J_{gauche} = 2.6$ Hz. These populations differ by <15% from those calculated using the ^3J values of Feeney (11).

Results of these calculations for the ABX spin system residues and for Lys[9] are given in Table IV. Because the β proton stereochemical assignments were not known, the population distributions shown for the 180° and −60° rotamers may be interchanged. Possibilities other than averaging among classical rotamers include (*a*) averaging of χ^1 among nonclassical rotamers, and (*b*) the existence of "frozen" conformations in which a single or narrow range of χ^1 values, either classical or nonclassical, pertains.

Classical analysis results indicated population of all three staggered rotamers for all of the somatostatin residues (Table IV). The most highly populated χ^1 state of any residue was $\chi^1 = 180°$ in Lys[9] with only 61%.

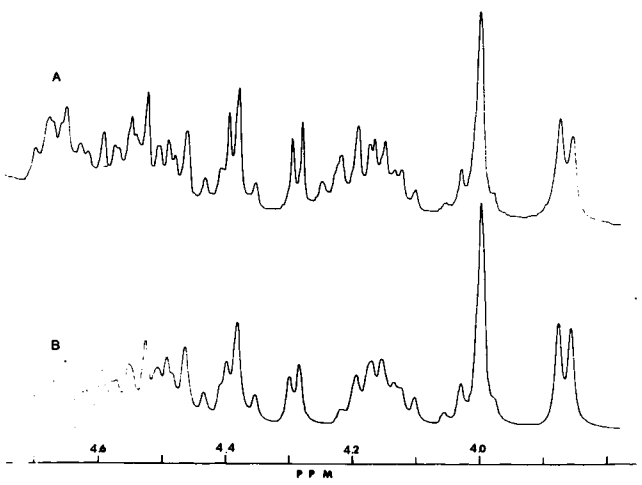

FIGURE 1 270 MHz, α region. (*A*) Somatostatin, observed. (*B*) Simulated spectrum of somatostatin, Lys[4] residue not included.

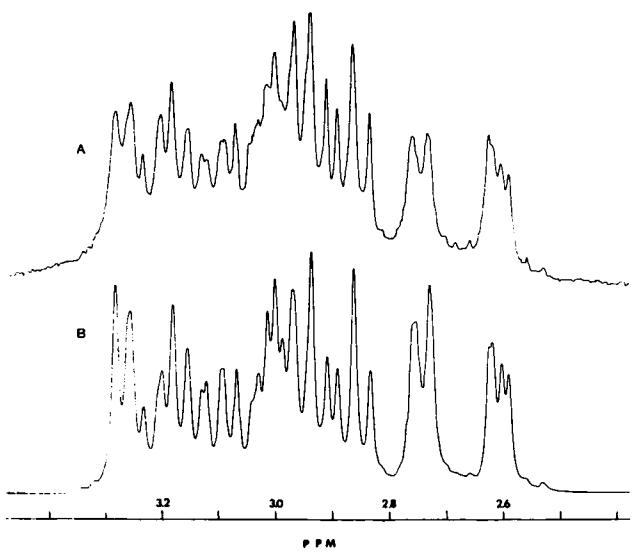

FIGURE 2 270 MHz, β region. (*A*) Somatostatin, observed. (*B*) Simulated spectrum of somatostatin.

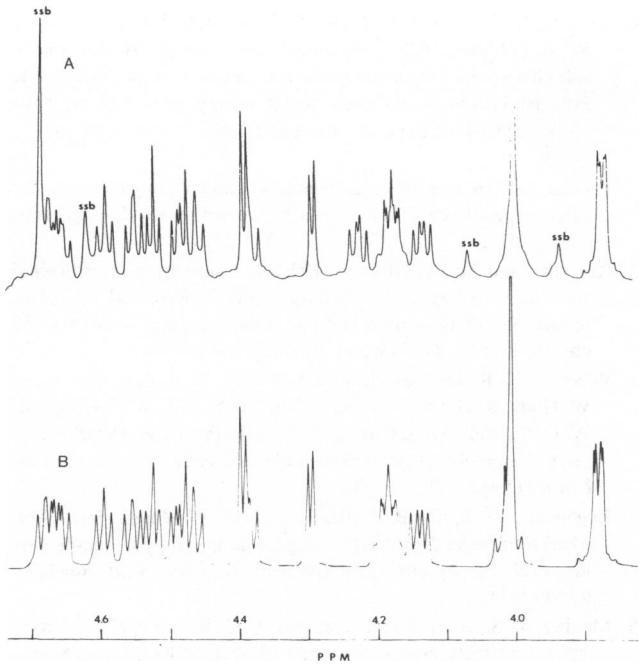

FIGURE 3 600 MHz, α region. (*A*) Somatostatin, observed. (*B*) Simulated spectrum of somatostatin, Lys[4] residue not included.

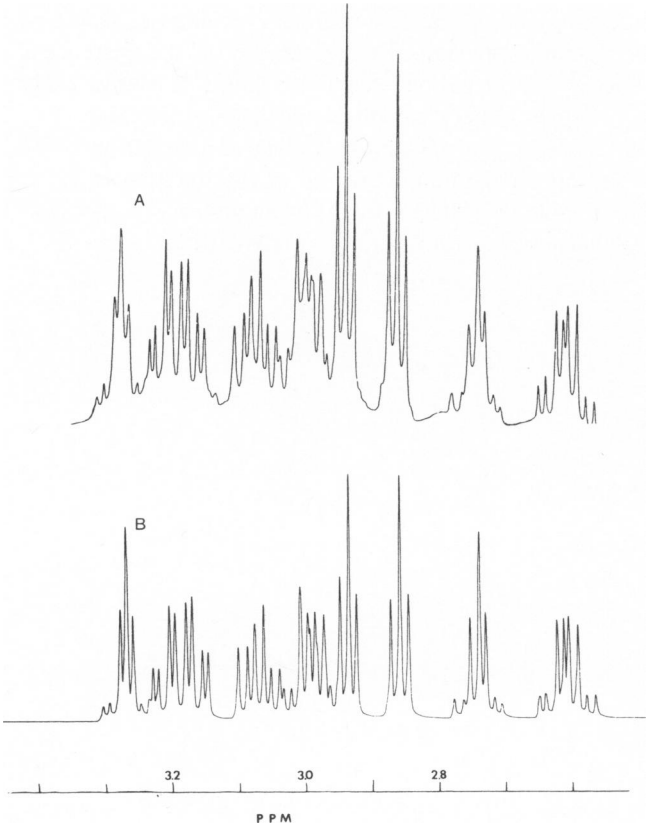

FIGURE 4 600 MHz, β region. (*A*) Somatostatin, observed. (*B*) Simulated spectrum of somatostatin.

TABLE IV
SIDE CHAIN ROTAMER POPULATIONS (IN PERCENT)

Residue	Populations		
	$\chi^1 = 180°$	$\chi^1 = -60°$	$\chi^1 = +60°$
Cys[3]	30	47	23
Asn[5]	29	49	22
Phe[6]	57	30	13
Phe[7]	36	49	15
Trp[8]	27	44	29
Lys[9]	61	25	14
Phe[11]	28	54	18
Ser[13]	29	23	48
Cys[14]	19	46	35

This is in contrast to the expectation for the side-chain rotamer conformations if one of the proposed β turn and sheets (4, 5) describes the predominant solution secondary structure of somatostatin. In both of these proposed secondary structures an aromatic residue faces another aromatic residue across a β pleated sheet (Phe[6] and Phe[11]) (4) or (Trp[8] and Phe[11]) (5). Steric considerations limit the pairs of allowable χ^1 values, thereby decreasing the degree of averaging. Aromatic residues are found in the β pleated sheet of tyrocidine A and classical rotamer analysis in that case showed strongly preferred χ^1 values, 86% population for $\chi^1 = 60°$ in D Phe[7] and 89–91% for $\chi^1 = -60°$ in Phe[6] (12).

The absence of highly preferred somatostatin χ^1 values (Table IV), especially for Phe[6], Trp[8], and Phe[11], suggests that somatostatin does not exist with one of the proposed β turn and sheet conformers as the predominant solution secondary structure. As with the similar conclusion above based on amide parameters, we cannot exclude averaging among various secondary structures which may include the proposed β structures.

A possible and interesting interpretation of the disparity we suggest between the secondary structure of somatostatin in solution and the β sheet and turn secondary structure of the highly potent synthetic analogue (4, 13) is that the secondary structure of the hormone bound to the receptor is not the dominant aqueous solution secondary structure of somatostatin.

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