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# ASSIGNMENT OF THE 270 MHz NUCLEAR MAGNETIC RESONANCE SPECTRUM OF SOMATOSTATIN IN WATER

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## SUMMARY

The proton nuclear magnetic resonance spectrum of the 14 residue peptide hormone somatostatin in $D_2O$ at 270 MHz has been assigned by comparing the spectra of synthetic analogs with those of the native peptide. Extensive difference double resonance studies of all somatostatins and pH titrations confirmed all assignments. $^3J_{NHCH}$ values and conventional NMR hydrogen bonding studies confirm the existence of preferred secondary conformations but not with a predominant conformation possessing a β-turn in either of the sequences 7-8-9-10 or 8-9-10-11. More extensive data treatment is needed before the actual conformation(s) of somatostatin is elucidated, but several NMR criteria for conformations are proposed.

## INTRODUCTION

Somatostatin is a recently discovered peptide hormone with the following structure:

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

Somatostatin inhibits the release of mammalian growth hormone, insulin and glucagon, and may act as a neurotransmitter (1). Studies of the biological activities of numerous synthetic analogs of somatostatin have suggested that the conformational properties of these molecules play an important role in determining both the potencies and specificities of their actions. The preferred conformation(s), however, has yet to be determined (1-4); a study of the conformation of somatostatin fragments has been described (5).

Nuclear magnetic resonance spectroscopy is an extremely powerful tool for investigating molecular conformation and specifically for that of

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peptides in solution (6,7). Prior to any detailed analysis of conformation by NMR (8) techniques, the observed resonances must be assigned to specific nuclei of the molecule; this is still not a trivial process for a 14 amino acid peptide.

We report here the assignment of the proton NMR spectrum of somatostatin. Scalar decoupling experiments were used to break the spectrum down into 14 sets of resonances, each of which is associated with one of the amino acid residues of somatostatin. A variety of methods enabled assignment of each group to a specific amino acid residue; these included identification of spin system types through scalar decoupling, comparison with chemical shift ranges characteristic of particular amino acid residues, scalar decoupling on somatostatin analogs, pH titration and nuclear Overhauser effect experiments.

## RESULTS AND DISCUSSION

The assigned proton NMR spectrum of somatostatin in aqueous solution at 270 MHz is shown in Figures 1-3. For the purpose of clarity the 14 sets of resonances are delineated in three parts, Figure 1 ($Gly^2$, $Thr^{10}$, $Thr^{12}$ and $Ser^{13}$), Figure 2 ($Lys^4$, $Lys^9$ and $Ala^1$) and Figure 3 ($Cys^3$, $Asn^5$, $Phe^6$, $Phe^7$, $Trp^8$, $Phe^{11}$ and $Cys^{14}$). Two important types of information are contained in these figures: the grouping of resonances into 14 sets and the identification of each of these with a specific amino acid residue of somatostatin.

### Assignment of Amino Acid Residue Resonances

The various resonances of the protons belonging to a single amino acid residue are connected to one another with heavy lines in Figures 1-3. These connections were determined by scalar decoupling experiments. Scalar coupling interactions are through-bond interactions which are predominantly between protons of a single amino acid residue. Coupling of protons of the aromatic rings ($Phe^6$, $Phe^7$, $Phe^{11}$ and $Trp^8$) to other protons is not observed; they were not assigned and are not shown in the figures.

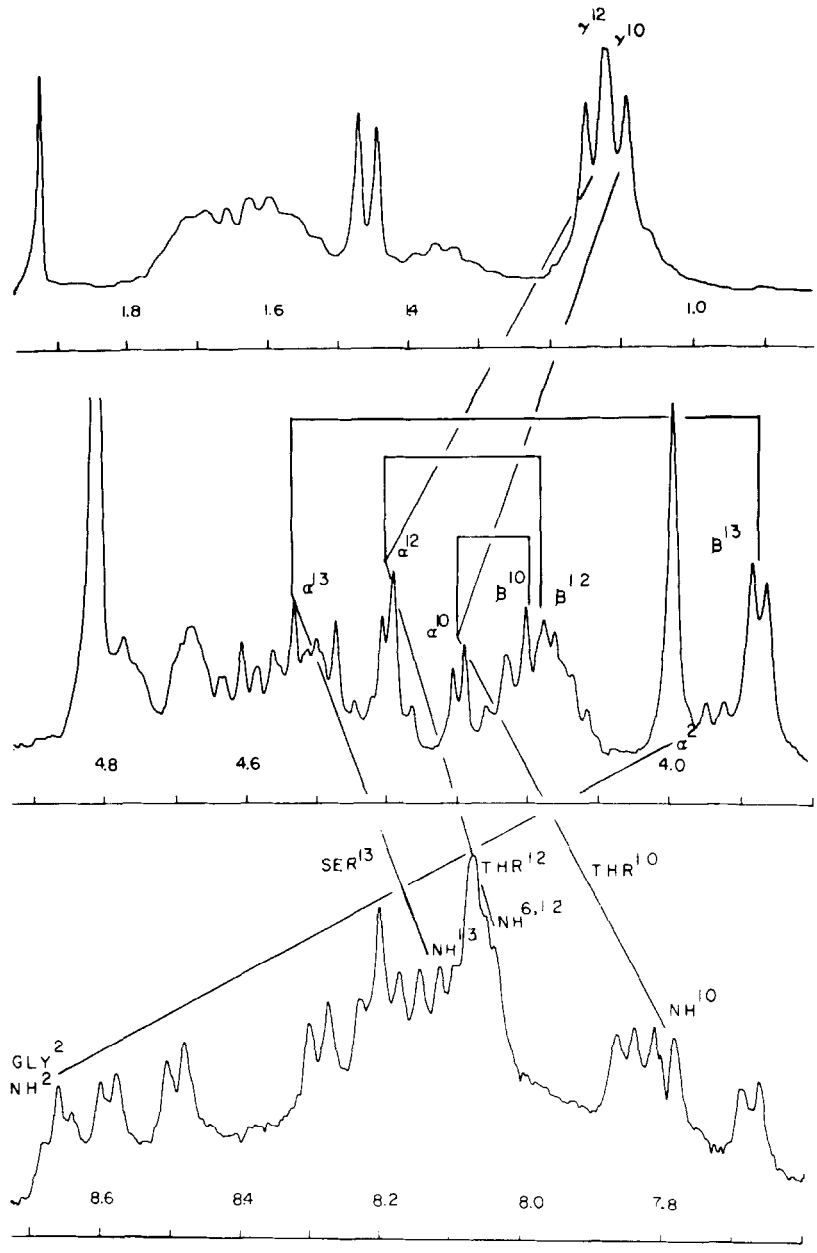


Figure 1. The assigned proton NMR spectrum of somatostatin at 26° C, 3 mM concentration. The regions above 4.8 p.p.m. were recorded in $D_2O$ at pD 6.5. The amide region (8.7 to 7.6 p.p.m.) was measured in $H_2O$ at pH 4.3. Scalar decoupling of amide protons in $H_2O$ solutions was accomplished with "under-water decoupling" using a steady-state water eliminated Fourier transform pulse (WEFT) sequence (14). These spectra were obtained on a Bruker Scientific WH270 MHz spectrometer interfaced with a Nicolet 1180 computer. An exponential line broadening function of 0.5 Hz was used. The various chemical shift regions of somatostatin are arranged vertically; the dark lines connect the resonances of the protons belonging to a given amino acid residue. The vertical scale for the chemical shift region of 0.9 to 1.8 p.p.m. is compressed by a factor of two relative to the other regions of the $D_2O$ spectrum. Figure 1 shows the resonances of $Thr^{10}$, $Thr^{12}$, $Ser^{13}$, and $Gly^{2}$.

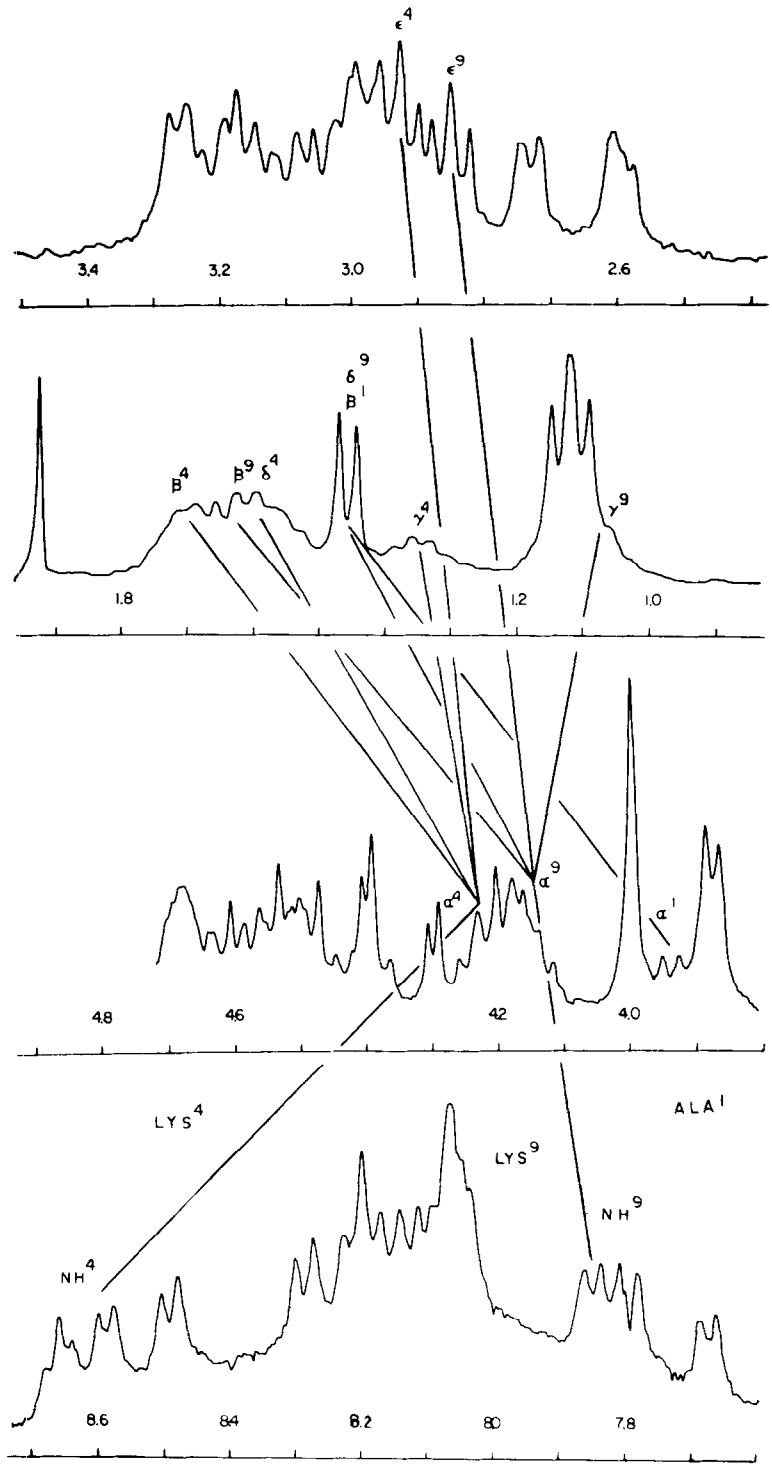


Figure 2 The resonances of amino acid residues $Lys^4$, $Lys^9$, and $Ala^1$. See Figure 1 caption for explanation.

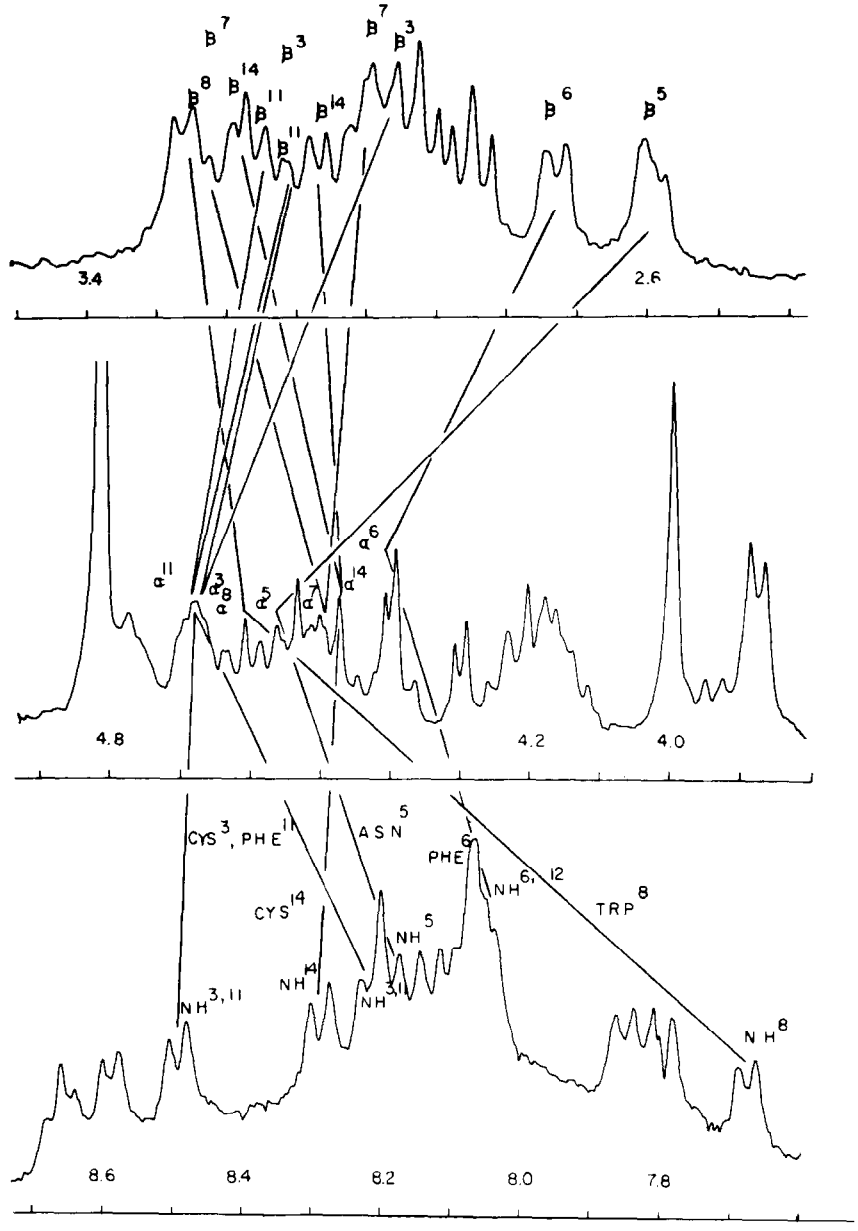


Figure 3 The resonances of amino acid residues Cys$^{3}$, Asn$^{5}$, Phe$^{6}$, Phe$^{7}$, Trp$^{8}$, Phe$^{11}$ and Cys$^{14}$. See Figure 1 caption for explanation.

The general pattern expected for the proton NMR of a given amino acid residue (chemical shift ranges, resonance intensities and number of resonances) is known, both from the type of spin system involved and from numerous measurements found in the literature of spectra of free amino

acids and peptides (6,7,9). On the basis of their patterns the resonances belonging to Ser, Thr and Gly (Figure 1) and to Lys and Ala (Figure 2) were identified. This approach did not afford complete assignment of these amino acid residues; a means of distinguishing $Lys^4$ from $Lys^9$ and $Thr^{10}$ from $Thr^{12}$ was also necessary. The resonances of the remaining seven amino acid residues (Figure 3, $Cys^3$, $Asn^5$, $Phe^6$, $Phe^7$, $Trp^8$, $Phe^{11}$ and $Cys^{14}$) all have the same general pattern.

Comparison of the proton NMR spectra and scalar decoupling spectra of somatostatin analogs enabled further assignment of the native somatostatin spectrum. The analogs employed were those in which an Ala residue replaces a somatostatin residue at a single position. The $A_3X$ spin pattern of the new Ala was easily recognized in the analog spectra. Types of changes other than the appearance of the Ala resonances and the loss of the resonances of the original amino acid may occur; due caution was exercised in using this method to make assignments.

Nine Ala-somatostatin analogs were examined. Eight of these, with substitutions at positions 4, 5, 6, 7, 10, 11, 12 and 13, were satisfactorily employed for assignment purposes while the $Trp^8 \rightarrow Ala^8$ analog differed too greatly from that of native somatostatin to be easily used for assignment purposes. The analog studies therefore allowed the distinguishing of $Thr^{10}$ from $Thr^{12}$, of $Lys^4$ from $Lys^9$ and the assigning of $Asn^5$, $Phe^6$, $Phe^7$, and $Phe^{11}$.

The amide and Cα proton resonances of the residue at the carboxy terminus of a peptide generally shift much more than any other amide and Cα proton resonances as the carboxyl group is titrated. Using these pH dependencies the $Cys^{14}$ amide and Cα proton resonances were identified and the $Cys^{14}$ residue resonances assigned (Figure 3).

The assignment of the resonances of one amino acid residue, $Cys^3$, was by process of elimination.

## Conformation from NMR Parameters

Our assignments of the proton NMR spectrum and evaluation of the proton NMR parameters should eventually permit a detailed elucidation of the solution conformation(s) of somatostatin and its analogs. We use the proton NMR parameters to: (1) resolve the question of whether or not somatostatin has a preferred or random coil conformation in aqueous solution, (2) evaluate published conformational models, and (3) state the proton NMR requirements that any model of somatostatin must satisfy.

The evidence that somatostatin possesses preferred rather than a random coil conformation comes from the following: (1) several $^{3}J_{NH-C\alpha H}$ values differ from the random coil value (12) of 7 Hz, (2) the amide protons of residues 8, 9 and 10 have relatively slow rates of exchange with solvent protons and low values of the temperature dependencies of the chemical shifts (13), and (3) many proton resonances have chemical shift versus guanidine hydrochloride concentration behavior similar to that discovered by circular dichroism (3) and consistent with cooperative denaturation, and (4) comparison of somatostatin residue proton chemical shifts with corresponding shifts in random coil model peptides (9).

Our data do not support the existence of the β turns proposed for somatostatin in aqueous solution (3) at positions 8-9-10-11, nor the β turn proposed for the hormone at its receptor site at positions 7-8-9-10 (4). Our evidence includes φ angles from experimental $^{3}J_{NH-C\alpha H}$ values for the corner residues of the proposed turns (J = 6.9, 6.3 and 8.3 for $Trp^{8}$, $Lys^{9}$ and $Thr^{10}$ respectively) and the absence of a characteristic β turn hydrogen bonding pattern for the amides. We cannot exclude the following possibilities: (1) conformational averaging occurs and a percentage of the existing conformers possess the proposed β turns, and (2) these β turns exist at the receptor and not in solution.

Any solution conformation(s) of somatostatin will have to incorporate the following data: (1) all φ angles derived from $^{3}J_{NH-C\alpha H}$ data (2) confor-

mational features in which the amides of residues 8, 9 and 10 are hydrogen bonded or solvent shielded, and (3) the chemical shift data including the deviations of chemical shifts from the Wuthrich values. Before correlation of solution spectra and conformational parameters with receptor activities and biological function can be achieved a detailed NMR study of several analogs of differing potencies and specificities is required.

## CONCLUSION

Assignment of the proton NMR spectrum has been achieved; the chemical shifts and $^{3}J_{NH-C\alpha H}$ values for amide and alpha backbone protons measured. These data and conventional proton NMR hydrogen bonding studies are consistent with the existence of a preferred conformation(s) in aqueous solution but inconsistent with a predominant conformation possessing $\beta$ turns within residues 7-11. NMR criteria for the actual conformation are proposed.

## ACKNOWLEDGEMENTS

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