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Conformational Studies on Somatostatin and Analogues†

Leslie A. Holladay,* Jean Rivier, and David Puett‡

ABSTRACT: Somatostatin is a 38-membered cyclic tetradecapeptide with the following structure: NH_2-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-COOH. In an effort to increase our knowledge of the conformational aspects of somatostatin in aqueous solution, and to understand better the individual contributions of the four aromatic residues to circular dichroic (CD) spectra and overall conformation, comparative physicochemical studies have been performed on analogues with the following replacements: for phenylalanine, D-Phe^6, D-Phe^7, and D-Phe^{11}; for tryptophan, Gly^8, Ala^8, D-Ala^8, and D-Trp^8. Also, [Leu^{12}]-somatostatin (i.e., replacement of Thr^{12}) was investigated as a derivative unable to form a hydrogen bond via its side chain. Based on the ability of the analogues to inhibit the spontaneous secretion of somatotropin from pituitary cells in vitro, the analogues exhibited varying potencies relative to somatostatin. [D-Trp^8]-Somatostatin was eightfold more potent; [D-Phe^6]-somatostatin, [D-Phe^{11}]-somatostatin, and [Leu^{12}]-somatostatin were about 5 to 15% as potent; and the other analogues were less than 1% as potent as somatostatin. Conformational studies were performed using near- and far-ultraviolet CD spectroscopy in buffered aqueous solution and in 6 M guanidinium chloride (GdmCl), a strong denaturing solvent. Ultracentrifugation, involving sedimentation equilibrium to ascertain monodispersity and an approach-to-equilibrium technique to determine diffusion constants with an estimated error of ±5%, was also employed. Diffusion constants were of particular importance since they permitted calculation of the frictional ratio, f/f_0, i.e., a measure of hydrodynamic asymmetry. The diffusion constants of somatostatin and the analogues ranged between 1.83 and 2.95 $\times 10^{-6}$ cm^2/s, corresponding to f/f_0's of 1.51 to 0.96. On the basis of direct CD spectra, solvent-induced difference CD spectra (i.e., between compounds in aqueous solution and the same with 6 M GdmCl), and frictional ratios, somatostatin and the analogues were grouped into three major classes. Somatostatin, [D-Phe^6]-somatostatin, and [Leu^{12}]-somatostatin appeared to have similar conformations and comparable asymmetries ($f/f_0 \simeq 1.1$-1.2). [D-Ala^8]-Somatostatin, [D-Trp^8]-somatostatin, and [D-Phe^{11}]-somatostatin seemed to have a greater degree of asymmetry ($f/f_0 > 1.3$) and an altered β bend as monitored by difference CD measurements. [Gly^8]-Somatostatin, [Ala^8]-somatostatin, and [D-Phe^7]-somatostatin appeared to have quite different conformations from somatostatin and widely different asymmetries. Based on model construction, these results are consistent with a proposed β bend in somatostatin. The average contribution of a single phenylalanyl residue to the CD spectrum of somatostatin in 6 M GdmCl was estimated to be +18 000 deg cm^2 dmol^{-1} (molar ellipticity) at 219 nm, with a rotational strength of $+1.22 \times 10^{-39}$ (cgs units). Under the same conditions, the contribution of Trp^8 was estimated to be +27 000 deg cm^2 dmol^{-1} (molar ellipticity) at 226 nm; the rotational strength of this band was $+1.47 \times 10^{-39}$ (cgs units). From these results and from the CD spectrum of somatostatin, it is possible to estimate the total (i.e., combined) contribution at 225 nm of the single disulfide and the thirteen peptide chromophores to the ellipticity of somatostatin in the denaturing solvent to be approximately −43 400 deg cm^2 dmol^{-1}. This represents the first study in which estimates of average aromatic contributions to the CD spectrum of a peptide greater than several residues have been obtained in the important spectral region of the peptide chromophore.

Since the original reports on the purification and sequence of ovine hypothalamic somatostatin (Brazeau et al., 1973; Burgus et al., 1973), numerous studies have appeared on the chemistry (Rivier, 1974), conformation (Holladay and Puett, 1975, 1976a), distribution and physiology (cf. Guillemin and Gerich, 1976; Vale et al., 1977) of this cyclic tetradecapeptide. In addition to the inhibition of somatotropin (Breazeau et al., 1973), somatostatin also inhibits the release of other pituitary hormones, the pancreatic hormones, and gut hormones (Vale et al., 1977). These release-inhibitory properties of somatostatin have proven quite useful in clarifying the role of particular hormones in glucose homeostasis (Guillemin and Gerich, 1976; Vale et al., 1977).

Due to the availability of a large number of synthetic somatostatin analogues (Rivier et al., 1976b), it became possible to attempt to refine a recently proposed conformation of somatostatin (Holladay and Puett, 1976a) with new physicochemical evidence. This work is concerned with CD[1] and hydrodynamic studies on synthetic somatostatin and eight (cy-

† From the Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37232 (L.A.H. and D.P.), and the Neuroendocrinology Laboratory, The Salk Institute, La Jolla, California 92037 (J.R.). *Received June 6, 1977.* This work was supported in large part by the National Institutes of Health (The Vanderbilt Diabetes-Endocrinology Center, AM-17026, and Research Grants AM-18811, AM-15838, and HD-09690). L.A.H. and D.P. also received partial support from the Vanderbilt University Research Council. D.P. was a Dreyfus Foundation Teacher-Scholar Awardee during the course of these studies, and National Foundation Grant 1-411 to Dr. R. Guillemin is gratefully acknowledged.

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[1] Abbreviations used: CD, circular dichroic; GdmCl, guanidinium chloride.

TABLE I: Biological Activity of Cyclic Somatostatin Analogues.[a]

Tetradecapeptide	% potency relative to somatostatin
Somatostatin	100
[D-Phe^6]-Somatostatin	5
[D-Phe^7]-Somatostatin	<1
[Gly^8]-Somatostatin	<1
[Ala^8]-Somatostatin	<0.5
[D-Ala^8]-Somatostatin	<1
[D-Trp^8]-Somatostatin	800
[D-Phe^{11}]-Somatostatin	10
[Leu^{12}]-Somatostatin	14

[a] Based on the inhibition of spontaneous somatotropin secretion from dispersed rat pituitary cells (cf. Rivier et al., 1976b).

clic) analogues. The analogues were chosen to provide information on the contribution of selected residues in somatostatin to CD spectra and conformation. The three Phe's at positions 6, 7, and 11 were each replaced with the D configuration, and the single Trp (position 8) was replaced with Gly, Ala, D-Ala, and D-Trp. Also, Thr^{12} was replaced with Leu in order to study a derivative lacking the ability to form stabilizing intramolecular hydrogen bond.

Materials and Methods

Spectrophotometric grade GdmCl and 2,2,2-trifluoroethanol were obtained from Heico, Inc., and Matheson Coleman and Bell, respectively. All other salts were reagent grade.

Peptide Synthesis and Characterization. Somatostatin and the various analogues were synthesized by solid phase, cyclized, purified, and chemically characterized as previously reported (Rivier, 1974; Rivier et al., 1975a,b, 1976a). Biological assays were based on the ability of somatostatin to inhibit the spontaneous secretion of somatotropin in primary cultures of enzymically dispersed rat anterior pituitary cells (Vale and Grant, 1975).

Circular Dichroism. The CD spectra were measured using a Cary 60 spectropolarimeter with a Model 6002 CD attachment equipped with a hollow cell block. The temperature was controlled at 25 ± 0.2 °C with a Lauda K2/R circulating temperature bath. Each spectrum represents the average of two to four scans. Concentrations of stock solutions were determined spectrophotometrically with extinction coefficients calculated using ϵ_{280} = 5600 M^{-1} cm^{-1} for tryptophanyl, ϵ_{280} = 110 M^{-1} cm^{-1} and ϵ_{257} = 300 M^{-} cm^{-1} for disulfides, and ϵ_{257} = 197 M^{-1} cm^{-1} for phenylalanyl residues (Sober, 1968). If necessary, corrections for light scattering were made using the standard method (Beaven and Holiday, 1952). Pathlengths of 0.5, 1, 5, and 10 mm were used for the CD scans. Unless otherwise indicated, $[\theta]$ represents the mean residue ellipticity in deg cm^2 $dmol^{-1}$. For the far-UV CD spectra, concentrations were about 0.2 mg/mL. For the near-UV CD spectra, concentrations were close to 1 mg/mL for somatostatin analogues lacking a tryptophanyl residue and about 0.2 mg/mL for analogues containing this aromatic residue.

Sedimentation Equilibrium. Data were obtained using a Model E ultracentrifuge equipped with electronic speed control and photoelectric scanner. For the peptides lacking a tryptophanyl residue, the monochromator and camera lens were set for 262 nm; otherwise 280 nm was used. Equilibrium runs were performed using the short column method of Van Holde and Baldwin (1958) with a 12-mm Kel-F centerpiece and AN-H rotor, using a solution column height of 2.7 mm. For all runs, somatostatin or the analogue was dissolved in 0.1 M KCl, 10 mM Tris-HCl, pH 7.0. Replicate scans using the slowest scan

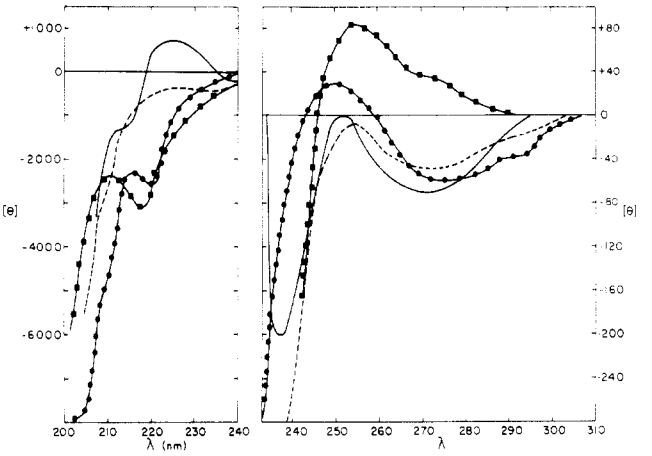

FIGURE 1: CD spectra of somatostatin (—), [D-Phe^{11}]-somatostatin (■—■), [Leu^{12}]-somatostatin (- - -), and [D-Phe^6]-somatostatin (●—●) in 0.1 M KCl, 10 mM Tris-HCl, pH 7, 25 °C.

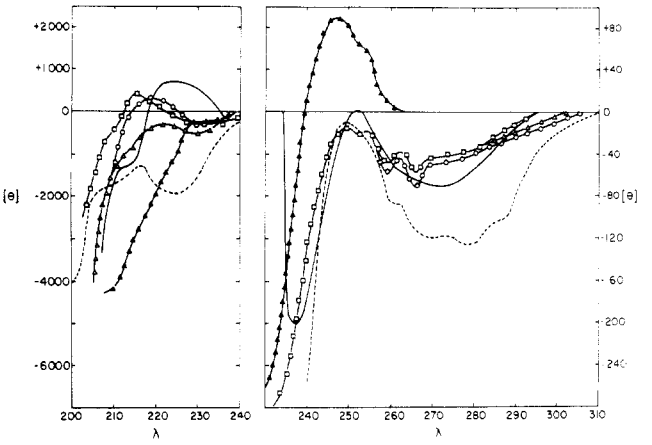

FIGURE 2: CD spectra of [D-Trp^8]-somatostatin (- - -), [Gly^8]-somatostatin (○—○), [Ala^8]-somatostatin (△—△), [D-Ala^8]-somatostatin (□—□), and [D-Phe^7]-somatostatin (▲—▲), compared with somatostatin (—). All peptides were in 0.1 M KCl, 10 mM Tris-HCl, pH 7, 25 °C. (Only a portion of the spectrum of [Ala^8]-somatostatin is shown since it overlaps almost exactly with the spectra of [Gly^8]-somatostatin and [D-Ala^8]-somatostatin.)

speed setting with 0.1-mm slit width were obtained after 11–12 h at 48 000 rpm, 20 °C. The ln (OD) vs. r^2 curves were analyzed by calculating the point apparent weight average molecular weight at 0.15-mm intervals and by fitting the entire curve to a parabola in r^2 and testing to determine if significant curvature existed in the plot. Partial specific volumes were calculated from the amino acid composition.

Approach to Sedimentation Equilibrium. The diffusion constant (D) was determined by the approach to equilibrium method (Van Holde and Baldwin, 1958; LaBar and baldwin, 1962). For the short column used in this study, the equations derived assuming a constant-field, rectangular boundary situation are very nearly exact. Plots of ln δ vs. t were obtained for t between about 300 and 4000 s. The intercepts, as calculated from the theoretical expression (Van Holde and Baldwin, 1958), and as computed by least-squares, were identical within experimental error. The diffusion constant was determined from the least-squares slope of the ln δ vs. t plot.

Results

Biological Potency of the Somatostatin Analogues. The potencies of the analogues, as measured by their ability to inhibit somatotropin release in vitro, are given in Table I. A more

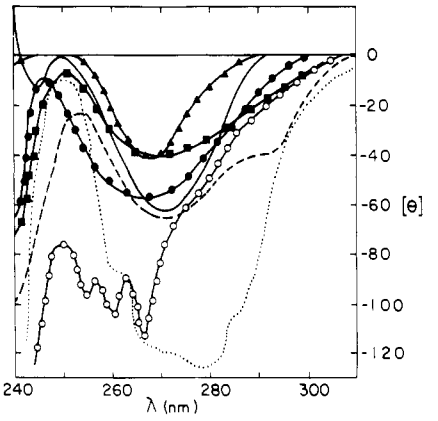

FIGURE 3: Near-UV CD spectra of somatostatin and analogues in 6 M GdmCl, 25 °C. The curves are labeled as in the legends to Figures 1 and 2; [D-Trp^8]-somatostatin is represented by the dotted line (···).

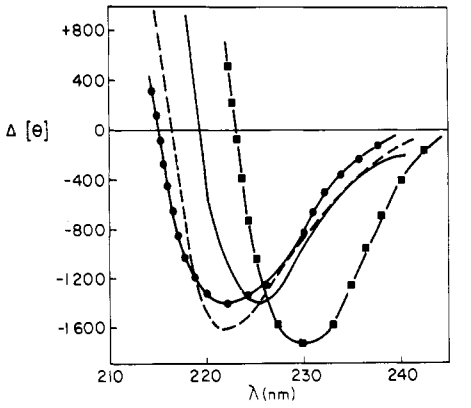

FIGURE 5: Computed CD difference spectra obtained by subtracting [θ] for peptides in 6 M GdmCl from [θ] in 0.1 M KCl, 10 mM Tris-HCl, pH 7, at each wavelength. Somatostatin (—), [Leu^{12}]-somatostatin (- - -), [D-Phe^6]-somatostatin (●—●), and [D-Phe^{11}]-somatostatin (■—■).

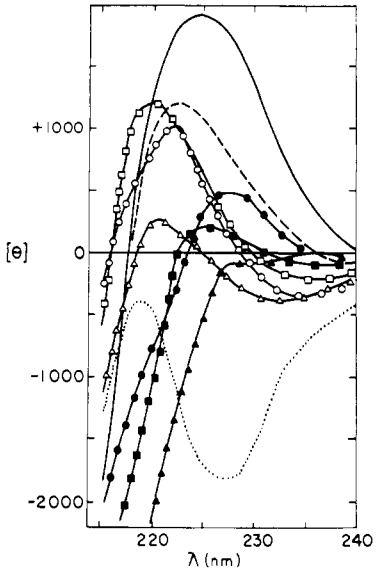

FIGURE 4: Far-UV CD spectra of somatostatin and analogues in 6 M GdmCl, 25 °C. The curves are labeled as in the legends to Figures 1 and 2.

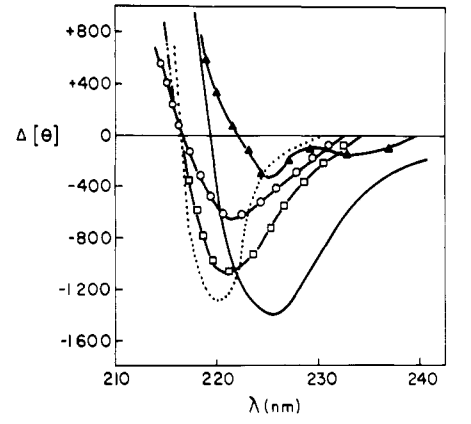

FIGURE 6: Computed CD difference spectra obtained by subtracting [θ] for peptides in 6 M GdmCl from [θ] in 0.1 M KCl, 10 mM Tris-HCl, pH 7, at each wavelength. The curves for [Ala^8]-somatostatin and [Gly^8]-somatostatin were nearly identical (○—○). Other curves are coded: somatostatin (—), [D-Phe^7]-somatostatin (▲—▲), [D-Trp^8]-somatostatin (···), and [D-Ala^8]-somatostatin (□—□).

detailed biological evaluation of these peptides, including their effects on the inhibition of glucagon and insulin release in vivo, has been reported elsewhere (Rivier et al., 1976b).

Circular Dichroism. The CD spectra of somatostatin (Holladay and Puett, 1975, 1976a) and of the eight analogues in 0.1 M KCl, 10 mM Tris-HCl, pH 7.0, are given in Figures 1 and 2. Five of the analogues possess near-UV CD spectra that resemble that of somatostatin. The CD spectra obtained for the analogues lacking a tryptophanyl residue have a much better signal-to-noise ratio, especially in the near-UV region, allowing clear resolution of the phenylalanyl bands. Both [D-Phe^6]-somatostatin and [D-Phe^7]-somatostatin exhibit positive ellipticity in the near UV. [D-Trp^8]-Somatostatin has a near-UV CD spectrum which is considerably elevated in absolute magnitude over that of somatostatin.

The CD spectra of the analogues in 6 M GdmCl are given in Figures 3 and 4. The three analogues with D-Phe replacements have very similar far-UV CD spectra. The analogues lacking a tryptophanyl residue have similar far-UV CD spectra, with the curves midway between that of somatostatin and [D-Trp^8]-somatostatin.

The calculated difference CD spectra, obtained for the peptides upon transfer from aqueous solutions to 6 M GdmCl solutions, are given in Figures 5 and 6. [Leu^{12}]-Somatostatin and [D-Phe^6]-somatostatin appear to have difference spectra nearly identical with that of somatostatin. [D-Trp^8]-Somatostatin and [D-Ala^8]-somatostatin have similar difference spectra which are blue shifted with respect to that of somatostatin. [Gly^8]-Somatostatin and [Ala^8]-somatostatin have markedly lower difference spectra, and the difference spectrum for [D-Phe^7]-somatostatin is essentially zero. [D-Phe^{11}]-Somatostatin has a difference spectrum which is some 5 nm red shifted with respect to that of somatostatin.

Sedimentation Equilibrium. Each peptide was examined by sedimentation equilibrium in order to test for monodispersity with regard to molecular weight, and Table II gives the results obtained. The M_w was determined from the least-squares slope of ln (C_r) vs. r^2. It is important to note that it was not possible to obtain the true baseline in the ultracentrifuge cell by overspeeding after the equilibrium scans due to the low molecular weight of the peptide. The slight discrepancies between M_w and the calculated molecular weight probably arise from small baseline errors. The agreement between the experimental and calculated molecular weight is quite good, with an average error of 3%. For all the peptides, the ln (OD) vs. r^2 plot appeared linear within experimental error; the coefficient of the $(r^2)^2$ term was not significantly different from zero for any of the peptides when the ln (OD) vs. r^2 data were fitted to a parabola in r^2.

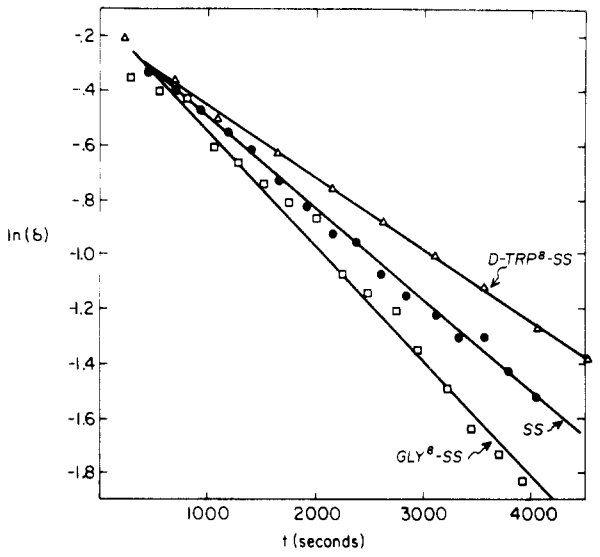

FIGURE 7: Representative plots obtained for diffusion constant measurements at 20 °C ($\delta = [\Delta C_{eq} - \Delta C_t]/\Delta C_{eq}$); SS denotes somatostatin.

Diffusion Constants. The approach-to-equilibrium method is capable of quite high precision when interference optics are used (Van Holde and Baldwin, 1958; LaBar and Baldwin, 1962); the problems arising from mixing during overlayering and with convection due to rotor precession (rotor speed 48 000 rpm), which can cause problems in the synthetic boundary method, are absent in this procedure. Figure 7 shows representative data for somatostatin and two analogues. Scanner optics give somewhat less precise data, but the error in determining δ [$=(\Delta C_{eq} - \Delta C_t)/\Delta C_{eq}$] is reasonably small. Table III gives the diffusion constants obtained with the approach-to-equilibrium method. The standard error (σ) column gives the error in $D_{20,w}$ which arises from the uncertainty in the ln δ vs. t plot. The observed differences between $D_{20,w}$ for the replicate runs which were done for three peptides are on the order of the error in determining the slope of the ln δ vs. t plot. These data suggest that the values of $D_{20,w}$ given in Table III are correct to within about 5%. Also presented in Table III are f/f_0 values for somatostatin and the analogues. These were calculated using standard methods.

Discussion

From the CD spectra (both direct and difference) and the hydrodynamic data, the analogues may be grouped into classes which reflect the degree and type of ordered secondary structure and the (hydrodynamic) asymmetry of the peptides. Inherent in this discussion are results from both theoretical calculations (Woody, 1974) and experimental studies (Urry et al., 1974) which suggest that the observed CD spectrum of a β bend is dependent upon the exact geometry of the bend, which in turn depends, in large part, upon the residues which become part of the β bend. Thus, as the β bend postulated for somatostatin (Holladay and Puett, 1975, 1976a) becomes perturbed, alterations are expected in the far-UV CD spectrum, both direct and difference. Moreover, similar far-UV CD spectra of analogues are at least tentatively interpreted as indicating similar overall conformations.

One class is composed of somatostatin, [Leu^{12}]-somatostatin, and [D-Phe^6]-somatostatin. These three tetradecapeptides exhibit virtually identical near-UV CD spectra and far-UV difference spectra and have similar f/f_0 values. Thus, their overall conformations appear to be similar.

TABLE II: Molecular Weight (M_w)[a] of Cyclic Somatostatin and Analogues.

Tetradecapeptide	C_0 (mg/mL)	M_{calcd}[b]	$\bar{V}$ (cm^3/g)	M_w/M_{calcd}
Somatostatin	0.09	1636	0.723	1.02
Somatostatin	0.13	1636	0.723	0.99
[D-Phe^6]-Somatostatin	0.10	1636	0.723	1.05
[D-Phe^7]-Somatostatin	0.09	1636	0.723	1.09
[Gly^8]-Somatostatin	0.55	1508	0.714	0.99
[Gly^8]-Somatostatin	0.55	1508	0.714	1.01
[Ala^8]-Somatostatin	0.50	1522	0.719	1.03
[Ala^8]-Somatostatin	0.46	1522	0.719	1.06
[D-Ala^8]-Somatostatin	0.43	1522	0.719	1.01
[D-Trp^8]-Somatostatin	0.17	1636	0.723	0.94
[D-Phe^{11}]-Somatostatin	0.10	1636	0.723	1.02
[Leu^{12}]-Somatostatin	0.16	1649	0.734	0.99

[a] Obtained from sedimentation equilibrium. [b] Calculated from the sequence of somatostatin and the analogues.

TABLE III: Diffusion Constants[a] of Cyclic Somatostatin and Analogues.

Tetradecapeptide	C (mg/mL)	$D_{20,w} \times 10^6$ (cm^2/s)	$\sigma_{D_{20,w}} \times 10^6$ (cm^2/s)	f/f_0
Somatostatin	0.09	2.45	0.06	1.13
Somatostatin	0.13	2.33	0.04	1.18
[D-Phe^6]-Somatostatin	0.10	2.34	0.08	1.18
[D-Phe^7]-Somatostatin	0.09	2.29	0.06	1.16
[Gly^8]-Somatostatin	0.55	2.95	0.10	0.96
[Gly^8]-Somatostatin	0.55	2.95	0.07	0.96
[Ala^8]-Somatostatin	0.50	2.14	0.09	1.32
[Ala^8]-Somatostatin	0.46	2.39	0.09	1.18
[D-Ala^8]-Somatostatin	0.43	2.04	0.09	1.39
[D-Trp^8]-Somatostatin	0.17	1.83	0.02	1.51
[D-Phe^{11}]-Somatostatin	0.10	2.03	0.05	1.36
[Leu^{12}]-Somatostatin	0.16	2.26	0.04	1.21

[a] Obtained from approach-to-sedimentation equilibrium.

Another class of analogues has an appreciable CD difference spectrum with a wavelength, corresponding to an ellipticity extremum, considerably removed from that of somatostatin. [D-Ala^8]-Somatostatin, [D-Trp^8]-somatostatin, and [D-Phe^{11}]-somatostatin fall within this class. These compounds appear to have an altered β bend resulting in a much greater degree of asymmetry, as reflected in f/f_0 values which cluster near 1.4.

The last class of analogues exhibits difference CD spectra appreciably lower than that of the other two classes. This may reflect the existence of a mixture of conformers for these analogues, i.e., [Gly^8]-somatostatin and [Ala^8]-somatostatin, in which β bends would be present in less than half the conformer population. [D-Phe^7]-Somatostatin in 80% 2,2,2-trifluoroethanol, a solvent favorable to β-bend formation (Urry et al., 1974), has a far-UV CD spectrum almost indistinguishable from that in buffer (data not shown). These data suggest that this somatostatin analogue does not form a β bend. [Gly^8]-Somatostatin has a f/f_0 value suggestive of a compact structure. The analogue, [Ala^8]-somatostatin, is indistinguishable from [Gly^8]-somatostatin insofar as the CD results are concerned; however, [Ala^8]-somatostatin appears to give somewhat different hydrodynamic behavior. This difference may result from the fact that both derivatives exist as a population of conformers, and that these are weighed differently

with regards to optical activity and hydrodynamic parameters.

An overall look at these results indicates that the substitutions at positions 7 (D-Phe) and 8 (Gly, Ala, D-Ala, and D-Trp) markedly affect somatostatin conformation, that substitution with D-Phe at position 11 has some effect, and that the substitutions at positions 6 (D-Phe) and 12 (Leu) have no major effect on conformation as examined by the methodologies used in this study. A β bend, with β-sheet structure involving residues 6 through 13, would explain the effects of substituting D-Phe residues for L residues. Hydrogen bonds from the CO of residue 6 to the NH of 13, from the NH of 8 to the CO of 11, and from the NH of 11 to the CO of residue 8, would give a structure in which D residues at positions 6 and 11 could be tolerated without completely altering the secondary structure, but for which a D residue at position 7 would place a phenyl ring in the center of the β-sheet area. There appears to be some type of interaction between the D-Phe^{11} side chain and Trp^{8} since the near-UV CD spectrum of [D-Phe^{11}]-somatostatin is markedly different from that of somatostatin. This model, which is obviously tentative, represents a refinement of a previously proposed model of somatostatin involving a β bend (Holladay and Puett, 1975, 1976a). The model explains the results of D for L substitutions of Phe and is consistent with the effects of substitutions at the other residues. Clearly, x-ray diffraction results are essential in order to obtain unambiguous results on this or any other model. It is of interest that results on infrared dichroism (γ-NH, amide I and amide II) and deuterium exchange of somatostatin and a few selected analogues in uniaxially oriented polyoxyethylene indicate that somatostatin exists in polyoxyethylene in a random conformation (Gilon, C., Simmons, D., Goodman, M., and Rivier, J., unpublished results).

In structure–function studies of peptides and proteins, it is always difficult to separate the effects of substituted or modified residues, leading to perhaps subtle conformational changes, from simple chemical alteration of specific and essential groups. Compounding the problem with somatostatin and many other biologically active peptides is the fact that binding parameters are unknown; i.e., it is not yet possible to discuss differences or similarities in residues involved in "receptor recognition" and "receptor activation". Also, the biological assays may reflect varying susceptibility of somatostatin and the analogues to distribution or enzymic degradation. In spite of these caveats, however, it is of interest to compare several of the analogues with somatostatin. In particular, somatostatin and the active analogue [D-Trp^{8}]-somatostatin deserve comment since, by our criteria, they differ somewhat in conformation. The increased potency of the analogue may reflect an increased stability with respect to enzymic degradation or possibly enhanced affinity for the receptor resulting from conformational effects. Another analogue, [D-Phe^{6}]-somatostatin, which is relatively inactive compared with somatostatin, apparently has a conformation which is at least similar to that of somatostatin. This could suggest an important role for the aromatic side chain of L-Phe at position 6 either in receptor recognition or activation. It is of interest to note that Phe at positions 7 and 11 may be replaced by Tyr without appreciable effects on biological activity, whereas a Tyr for Phe substitution at position 6 results in a derivative with markedly lower potency (Rivier et al., 1976b). More definitive interpretations of the structure–activity results must await clarification of somatostatin conformation and a better understanding of the peptide–receptor interaction.

Using the CD data for somatostatin and the analogues in 6 M GdmCl, a strong denaturing solvent (Pace, 1975) which is expected to disrupt any ordered secondary structure or a stable tertiary structure in somatostatin (Holladay and Puett, 1976a), it is possible to compute the contribution of Phe and Trp to the far-UV CD of somatostatin in the denaturing solvent. The CD difference spectrum generated by subtracting $[\theta]$ for [D-Ala^{8}]-somatostatin from that of [D-Trp^{8}]-somatostatin (both in 6 M GdmCl) is essentially a mirror image of that obtained by subtracting $[\theta]$ for [Ala^{8}]-somatostatin from that of somatostatin (both in 6 M GdmCl). The estimated maximum contribution of the single Trp to the CD of somatostatin in 6 M GdmCl is calculated from these data to be +27 000 deg cm^2 dmol^{-1} (molar ellipticity) at 226 nm, with a corresponding rotational strength (R) = 1.47×10^{-39} (cgs units). These data are in excellent agreement with our previous findings on Trp contributions to CD using the tetrapeptide, Gly-Trp-Gly-Gly (Holladay and Puett, 1976b). In 6 M GdmCl, the Trp contribution to $[\theta]$ at 221 nm was found to be +24 000 deg cm^2 dmol^{-1} (molar ellipticity) and the corresponding rotational strength was 1.5×10^{-39} (cgs units).

Similarly, the average contribution of a single Phe to the CD of somatostatin in 6 M GdmCl can be computed by subtracting the average of the CD spectra in 6 M GdmCl for [D-Phe^{6}]-somatostatin, [D-Phe^{7}]-somatostatin, and [D-Phe^{11}]-somatostatin from the CD spectrum of somatostatin in 6 M GdmCl and dividing by two. The contribution of a single Phe is calculated to be +18 000 deg cm^2 dmol^{-1} (molar ellipticity) at 219 nm, with $R = 1.22 \times 10^{-39}$ (cgs units). Using the tripeptide, Gly-Phe-Gly, in 6 M GdmCl, we previously found a $[\theta]$ contribution of Phe to be +18 000 deg cm^2 dmol^{-1} (molar ellipticity) with $R = 0.92 \times 10^{-39}$ (cgs units) (Holladay and Puett, 1976b).

The estimated contribution of all the aromatic residues to the CD of somatostatin in 6 M GdmCl at 225 nm is calculated to be nearly +70 000 deg cm^2 dmol^{-1} (molar ellipticity), or +5000 deg cm^2 dmol^{-1} (mean-residue ellipticity). Using these values and the experimentally observed $[\theta]$, the combined contribution of the single disulfide and the 13 peptide chromophores to the CD spectrum of somatostatin in 6 M GdmCl at 225 nm can be calculated to be about −43 400 deg cm^2 dmol^{-1} (molar ellipticity), or −3100 deg cm^2 dmol^{-1} (mean-residue ellipticity).

The overall agreement on aromatic contributions to peptide CD spectra between this investigation on cyclic tetradecapeptides, and the earlier study on tripeptides and tetrapeptides (Holladay and Puett, 1976b), is indeed remarkable. Studies such as these are essential for elucidation, on an empirical basis, of the role of conformation to the CD contributions of aromatic side chains. Hopefully, it will eventually be possible to obtain even more definitive information from CD spectroscopy on the conformation of linear and cyclic peptides, and the microenvironments of aromatic groups.

Lastly, this study has demonstrated the general utility of the analytical ultracentrifuge for determining diffusion constants of cyclic peptides.

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Purification of an Acetylcholine Receptor from a Nonfusing Muscle Cell Line†

Jim Boulter* and Jim Patrick

ABSTRACT: A nicotinic acetylcholine receptor has been solubilized and purified from the nonfusing, mouse muscle cell line BC_3H1. The presence of an acetylcholine receptor was monitored throughout the purification by the specific binding of α-[^{125}I]bungarotoxin. Affinity chromatography of Triton X-100 solubilized BC_3H1 membranes on an agarose-α-neurotoxin conjugate followed by sucrose density gradient centrifugation resulted in a 1700-fold purification of the α-[^{125}I]bungarotoxin binding component with a final specific activity of 2600 pmol of α-[^{125}I]bungarotoxin binding sites per mg of protein. The purified acetylcholine receptor has an apparent sedimentation coefficient of 9.5×10^{-13} s, is a glycoprotein containing glucosyl and/or mannosyl residues but no detectable D-fucose, D-galactose, or *N*-acetyl-D-glucosamine, and shares some antigenic determinants with acetylcholine receptors purified from *Electrophorus electricus* and *Torpedo californica*. Polyacrylamide gel electrophoresis of the purified acetylcholine receptor in the presence of sodium dodecyl sulfate revealed four subunits with apparent molecular weights of 72 000, 65 000, 53 000, and 44 000. A radioactive preparation of purified acetylcholine receptor has been obtained from BC_3H1 cells cultured in the presence of [^{3}H]leucine. Sucrose density gradient analysis of the [^{3}H]acetylcholine receptor demonstrates that all of the α-[^{125}I]bungarotoxin binding activity and 95% of the [^{3}H]leucine radioactivity migrate as a single symmetrical peak with an apparent sedimentation coefficient of 9.5×10^{-13} s. Evidence is presented which suggests that the purified acetylcholine receptor preparation contains a mixture of both "surface" and "hidden" receptor populations. Finally, a rabbit anti-BC_3H1 acetylcholine receptor antiserum has been prepared.

The acetylcholine receptor (AcChR)[1] has now been purified and characterized by a number of laboratories from a variety of sources (Miledi et al., 1971; Biesecker, 1973; Schmidt and Raftery, 1973; Chang, 1974; Lindstrom and Patrick, 1974; Meunier et al., 1974; Weill et al., 1974, Merlie et al., 1975; Brockes and Hall, 1975a). Although a vast literature concerning the physicochemical nature and general pharmacology of the AcChR from these sources is available, less is presently known concerning the cellular biochemistry of the AcChR. Recently, however, considerable progress has been made in this area utilizing organ and cell culture techniques (Brockes and

† From the Department of Neurobiology, The Salk Institute, San Diego, California 92112. *Received May 3, 1977.* J.B. was supported by National Institutes of Health Postdoctoral Fellowships 1 F22 NS00246 and 1 F32 NS05669. This work was supported by grants from the National Institutes of Health and the Muscular Dystrophy Associations of America, Inc., to J.P.

[1] Abbreviations used are: α-[^{125}I]BuTx, α-[^{125}I]bungarotoxin; α-NTx, α-neurotoxin; AcCh, acetylcholine; AcChR, acetylcholine receptor; PBS (phosphate-buffered saline), 138 mM NaCl, 3 mM KCl, 0.9 mM $CaCl_2$, 0.05 mM $MgCl_2$, 8.15 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.2; 0.5% PBS, PBS containing 0.5% (w/v) Triton X-100; 3% PBS, PBS containing 3% (w/v) Triton X-100; dTC, *d*-tubocurarine; Prep medium, Dulbecco modified Eagle's medium in which the bicarbonate is replaced with 1.08 mM Na_2HPO_4–1.5 mM K_2HPO_4; BSA, bovine serum albumin; Na-$DodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; Con A, concanavalin A.