Secretin. VI.[1] Simultaneous "in Situ" Syntheses of Three Analogues of the C-Terminal Tricosapeptide and a Study of Their Conformation

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Abstract: The tricosapeptide corresponding to the C-terminal sequence of secretin, S_{5-27}, and three analogues in which side-chain carboxyl groups are replaced by carboxamides, 9-Gln-S_{5-27}, 15-Asn-S_{5-27}, and 9-Gln-15-Asn-S_{5-27}, were prepared. They were synthesized simultaneously via stepwise chain lengthening with active esters, by the "in situ" technique. A comparison of the CD spectra of aqueous solutions of the four tricosapeptides provided evidence for the participation of ion pairs in the stabilization of the secondary-tertiary structure of secretin. The salt bridges were found to contribute in a cooperative way to a defined architecture.

The discovery of the gastrointestinal hormone secretin by Bayliss and Starling[3] in 1902 was followed by the isolation[4] of the pure peptide and the elucidation[5] of its structure by Jorpes, Mutt, and Magnusson. The sequence of the 27 amino acid residues (Figure 1) was proved by synthesis.[6] Conformational studies by ORD-CD[1,7] and by NMR spectra[8] revealed that the single chain of secretin has, in aqueous solutions, a folded[9] and partially helical structure.[10] It was not a priori obvious that a relatively short peptide chain should have a preferred conformation. The recognition that secretin can be considered a miniature protein with a defined architecture prompted further investigations into the nature of the intramolecular forces that cause folding of peptide chains.

Discussion

Our earlier studies demonstrated that, in secretin, folding and helicity are dependent on each other,[7] and thus the geometry of the molecule is determined by long-range, cooperative interactions. In the present paper, we report the results of experiments designed to examine the possible role of *ion-pair formation* in folding. The sequence of the hormone (Figure 1) contains four arginine residues with readily protonated guanidino groups. There are three acidic residues in the sequence: aspartic acid in positions 3 and 15, and glutamic acid in position 9. Of these, the aspartyl residue in position 3 clearly plays no role in the stabilization of the conformation: the C-terminal tricosapeptide (S_{5-27}) exhibits ORD-CD spectra that are almost identical with those of the entire chain.[7] On the other hand, it was conceivable that the glutamyl residue and/or the aspartyl residue in position 15 participate in the formation of intramolecular ion pairs ("salt bridges") such as the ones shown in Figure 2. For these reasons, we decided to synthesize and to study analogues of the C-terminal tricosapeptide, S_{5-27}, in which these acidic residues are replaced by amino acids with non-ionizable side chains. In order to keep the modification of the parent molecule to a minimum, asparagine was selected to replace aspartic acid and glutamine to substitute for glutamic acid. In this way, the shape, size, and polar character of the side chains probably remain unaltered, while their ion-forming ability is eliminated. Therefore, three analogues of S_{5-27}, namely 9-glutamine-S_{5-27}, 15-asparagine-S_{5-27}, and 9-glutamine-15-asparagine-S_{5-27}, were synthesized. As a control of the synthetic procedure, the unaltered tricosapeptide S_{5-27} was also prepared.

For the synthesis of the four tricosapeptides, the stepwise strategy[11] was applied. The procedure used in the first synthesis of secretin was followed, except that *o*-nitrophenyl esters[12] rather than *p*-nitrophenyl esters[13] were employed for the incorporation of most of the amino acid residues. Also, in some steps, acylation by active esters was catalyzed by the addition of 1-hydroxybenzotriazole.[14] From the dodecapeptide stage on, the syntheses were facilitated by the application of the "in situ" technique,[15] and it became possible to carry out the preparation of the four tricosapeptides[16] *simultaneously*.

Experimental Section

Capillary melting points are reported uncorrected. The solvents were reagent grade; DMF was stored over a Linde 4XA molecular sieve. For TLC, precoated silica gel plates (Merck) and microcrystalline cellulose plates (Analabs) were used with the following systems: (A) *n*-BuOH-AcOH-H_2O (4:1:1); (B) *n*-BuOH-Pyr-AcOH-H_2O (15:12:3:10); (C) *n*-BuOH-Pyr-AcOH-H_2O (30:12:3:10); uv, ninhydrin spray, fluorescamine[17] spray (2 mg of fluorescamine in 10 ml of acetone), chlorination,[18] charring,[19] and the Sakaguchi reaction were used for detection. For quantitative amino acid analysis, samples were hydrolyzed with constant boiling HCl in evacuated, sealed ampoules at 110 °C for 16 h, and the ratio of amino acids determined on a Beckman-Spinco 120C instrument according to the method of Spackman, Stein, and Moore.[20] For enzymatic hydrolysis, aminopeptidase M (Röhm) was applied;[21] because of lack of separation, Gln and Asn were not determined.

For analysis by fluorescence of countercurrent distributions, a Hitachi Perkin-Elmer fluorescence spectrophotometer MPF-2A was used. Aliquots from each tube were diluted to 3.0 ml with a 0.1 M phosphate buffer (pH 8.4) and mixed with 1.0 ml of fluorescamine reagent (2 mg per 10 ml of acetone). The excitation wavelength was set at 390 nm and relative fluorescence read at 480 nm.

All "in situ" operations,[15] including coupling, deblocking, and isolation of intermediates, were carried out in 40-ml glass centrifuge tubes fitted with standard 24/40 ground-glass tapered joints.

Benzyloxycarbonyl-L-leucyl-L-leucyl-L-glutaminylglycyl-L-leucyl-L-valinamide (I). The protected pentapeptide intermediate was prepared essentially as described before,[6] except that *o*-nitrophenyl esters[12] rather than their para analogues were applied for the incorporation of benzyloxycarbonylglutamine and, at the pentapeptide stage, for benzyloxycarbonylleucine. The results were comparable with those reported earlier.[6] After deprotection of the pentapeptide derivative, acylation with benzyloxycarbonylleucine *o*- and *p*-nitrophenyl ester was catalyzed with an equivalent amount of 1-hydroxybenzotriazole.[14] Parallel experiments demonstrated the efficacy of this catalyst. Without catalysis, several hours were needed for completion of the acylation. In the presence of 1-hydroxybenzotriazole, the ninhydrin reaction was negative after 0.5 h. The products were comparable by melting point (264-266 °C dec) and TLC ($R_f A$ 0.75); $[\alpha]^{25}D$ −43 °C (*c* 2, AcOH). Amino acid analysis: Glu, 1.0; Gly, 1.0; Val, 1.0; Leu, 3.1.

Anal. Calcd for $C_{38}H_{62}N_8O_9$: C, 58.9; H, 8.1; N, 14.4. Found: C, 58.7; H, 8.1; N, 14.5.

HIS-SER-ASP-GLY-THR-PHE-THR-SER-GLU-LEU-SER-ARG-LEU-ARG
1 2 3 4 5 6 7 8 9 10 11 12 13 14

ASP-SER-ALA-ARG-LEU-GLN-ARG-LEU-LEU-GLN-GLY-LEU-VAL-NH_2
15 16 17 18 19 20 21 22 23 24 25 26 27

Figure 1. The amino acid sequence of porcine secretin.

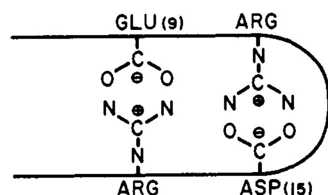

Figure 2. Possible salt bridges in secretin.

N-Butyloxycarbonyl-O-benzyl-L-seryl-L-alanylnitro-L-arginyl-L-leucyl-L-glutaminylnitro-L-arginyl-L-leucyl-L-leucyl-L-glutaminylglycyl-L-leucyl-L-valinamide (II). The protected hexapeptide I was treated with HBr in acetic acid and acylated in the presence of triethylamine with benzyloxycarbonylnitro-L-arginine-2,4-dinitrophenyl ester.[22] For the incorporation of the subsequent residues, with the exception of arginine, *o*-nitrophenyl esters[12] of benzyloxycarbonyl amino acids were used. The last amino acid added, *O*-benzyl-L-serine, was protected by the *t*-butyloxycarbonyl group. To reduce the time needed for coupling and thereby to keep the formation of a pyroglutamyl peptide at a minimum, the leucine residue was introduced in the presence of the catalyst 1-hydroxybenzotriazole (1 equiv). Acylation with benzyloxycarbonyl-L-alanine was catalyzed likewise. Otherwise, the synthesis closely followed the one described earlier.[6] The protected dodecapeptide amide appeared as a single spot on TLC (R_fA 0.42), mp 250-260 °C (lit.[6] 245-248 °C). Amino acid analysis: Ser, 0.80; Glu, 2.0; Gly, 1.0; Ala, 1.0; Leu, 3.8; Val, 1.0; Arg, 1.8; NH_3, 3.2.

Anal. Calcd for $C_{71}H_{121}N_{23}O_{21}$: C, 52.2; H, 7.5; N, 19.7. Calcd for $C_{71}H_{121}N_{23}O_{21}\cdot H_2O$: C, 51.7; H, 7.5; N, 19.5. Found: C, 52.0; H, 7.6; N, 19.2.

Protected Tricosapeptides. Four portions (0.78 g each) of compound II were placed in four 40-ml centrifuge tubes. After deprotection with trifluoroacetic acid (TFA), the chain was lengthened by acylation with active esters. In a typical cycle, the protected tetradecapeptides Boc-Arg(NO_2)-Asp(Bzl)-Ser(Bzl)-Ala-Arg-(NO_2)-Leu-Gln-Arg(NO_2)-Leu-Leu-Gln-Gly-Leu-Val-NH_2 and Boc-Arg(NO_2)-Asn-Ser(Bzl)-Ala-Arg(NO_2)-Leu-Gln-Arg-(NO_2)-Leu-Leu-Gln-Gly-Leu-Val-NH_2 were treated with 99% TFA (5 ml per tube) for 15 min at room temperature. The TFA was removed in vacuo, the residues were triturated with ether (30 ml per tube) and centrifuged, and the solid product was washed twice with ether (20 ml per washing per tube). The trifluoroacetate salts were dried in vacuo over NaOH. Small samples were examined on TLC (system A). The partially deprotected tetradecapeptides were dissolved in DMF (about 9 ml per tube); a Thermolyne-Max-Mix shaker was used to facilitate dissolution. Triethylamine was added dropwise until an alkaline reaction was detected in the vapor phase above the surface of the solution with moist indicator paper. Boc-Leu-ONO,[12,15] enough to produce a 0.1 M solution, was added, followed by an equimolar amount of 1-hydroxybenzotriazole. More triethylamine was added, just enough to maintain the slight alkalinity of the reaction mixture. The acylation was complete (negative ninhydrin and fluorescamine spot tests) within 2 h. The solvent was removed in vacuo, and the residues were treated with EtOAc (30 ml per tube). The precipitates were separated by centrifugation, washed four times with EtOAc (30 ml per tube each time), dried in air, and finally in a desiccator over P_2O_5. Quantitative yields were obtained in this particular step, and yields better than 95% were observed in each chain-lengthening cycle. Small samples were removed for quantitative amino acid analyses, and the syntheses were continued. For the incorporation of threonine, *t*-butyloxycarbonyl-L-threonine-2,4-dinitrophenyl ester[6] was applied. Melting points and R_fA values of the protected intermediates are listed in Table I.

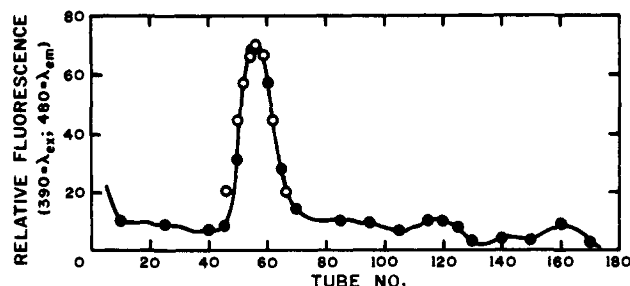

Figure 3. Countercurrent distribution (200 transfers) of the tricosapeptide 9-Gln-15-Asn-S_{5-27}: (●) experimental values; (○) calculated values (K = 0.4).

Free Tricosapeptides. The deprotection and purification of 9-Gln-15-Asn-S_{5-27} is described here as a typical example. A sample (200 mg) of the protected tricosapeptide was treated with TFA (1.0 ml) at room temperature for 15 min. Ether (9 ml) was added, and the precipitate was centrifuged, washed with ether, and briefly dried in air. The product was dissolved in 80% acetic acid (30 ml) and hydrogenated in the presence of a Pd-black catalyst[23] for 40 h. After the catalyst and solvent were removed, the residue was dissolved in a small volume of H_2O (5 ml) and lyophilized. The reduction of nitro groups was ascertained by examination of the uv spectrum.

The crude peptide was dissolved in both layers (2 ml each) of the solvent system *n*-butanol-0.1 M phosphate buffer (pH 7) and distributed[24] in an automatic Craig instrument (3 ml of each phase per tube) through 200 transfers. The distribution of the peptide was first scanned by spot tests and then determined quantitatively, with fluorescamine, on 0.2-ml aliquots taken from every 5th tube, from tube No. 40 to tube No. 70. The distribution curve is shown in Figure 3. The contents of tubes 47-67 were pooled and diluted with ethanol (60 ml), and the solution was stored in the cold (5 °C) overnight. The bulk of the phosphates was removed by filtration, and the filtrate was diluted with water (to a volume of 3 l.), acidified to pH 3, and treated with alginic acid (E. Mendell Co.) (2.5 g).[24] The alginic acid was collected on a filter, washed with ice-cold 0.001 N HCl (5 ml), and eluted with 0.2 N HCl (18 ml).

Table I. Decomposition Points and R_f Values (TLC) of Protected Intermediates

Protected peptide	Decomposition points[a]					R_fA values (TLC, silica gel)			
	9-Glu, 15-Asp Lit.[6]	9-Glu, 15-Asp	9-Gln, 15-Asp	9-Glu, 15-Asn	9-Gln, 15-Asn	9-Glu, 15-Asp	9-Gln, 15-Asp	9-Glu, 15-Asn	9-Gln, 15-Asn
Tridecapeptide	308	320		320		0.51		0.41	
Tetradecapeptide	300	310		310		0.48		0.39	
Pentadecapeptide	245	245		230		0.46		0.37	
Hexadecapeptide	240	241		240		0.51		0.46	
Heptadecapeptide	240	248		245		0.52		0.48	
Octadecapeptide	305	320		320		0.55		0.55	
Nonadecapeptide	315	325	320	328	339	0.52	0.49	0.48	0.46
Eicosapeptide	320	323	322	325	323	0.54	0.51	0.53	0.50
Heneicosapeptide	315	328	318	328	328	0.53	0.51	0.52	0.48
Docosapeptide	315	324	324	324	324	0.61	0.59	0.59	0.58
Tricosapeptide	310	326	326	327	327	0.60	0.59	0.60	0.58

[a] The decomposition points were found to be dependent on rate of heating and decomposition occurred gradually.

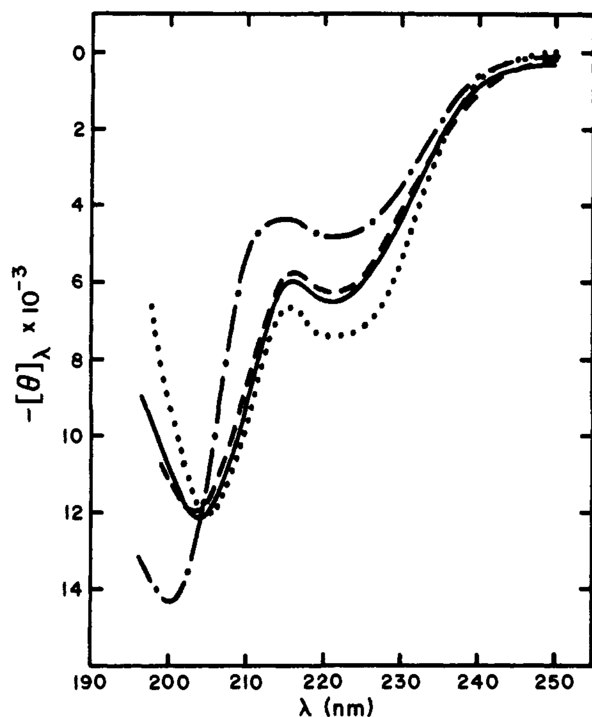

Figure 4. CD spectra (in H_2O) of tricosapeptides: (···) S_{5-27}; (- - -) 9-Gln-S_{5-27}; (—) 15-Asn-S_{5-27}; (· — · —) 9-Gln-15-Asn-S_{5-27}.

The eluate was passed through a column (1.25 × 22 cm) of DEAE-Sephadex in the acetate cycle, and the chloride-free solution was lyophilized. The peptide content of the product as calculated from the recovery of the amino acid analysis was 22 mg: Asp, 0.9; Thr, 1.7; Ser, 2.8; Glu, 3.0; Gly, 1.1; Ala, 0.9; Val, 1.0; Leu, 6.1; Phe, 0.9; NH_3, 5.2; Arg, 4.0. Enzyme hydrolysis: NH_3, 1.1; Arg, 4.0; *Asp, 0.0;* Thr, 1.8; Ser, 3.0; *Glu, 0.0;* Gly, 1.0; Ala, 1.1; Val, 0.9; Leu, 6.3; Phe, 0.9.

9-Gln-S_{5-27} was purified similarly. The *K* value (because of mechanical difficulties, only approximate) is about 0.9. Amino acid analysis: Asp, 0.9; Thr, 1.7; Ser, 2.5; Glu, 3.0; Gly, 1.0; Ala, 1.0; Val, 0.9; Leu, 5.9; Phe, 0.9; NH_3, 4.5; Arg, 4.0. Enzyme hydrolysis: NH_3, 1.9; Arg, 4.0; Asp, 1.0; Thr, 2.0; Ser, 3.0; *Glu, 0.0;* Gly, 1.0; Ala, 1.2; Val, 0.9; Leu, 6.0; Phe, 0.7.

15-Asn-S_{5-27} was purified as described above. The *K* value (because of mechanical difficulties, only approximate) is about 0.7. Amino acid analysis: Asp, 1.1; Thr, 2.0; Ser, 2.9; Glu, 3.0; Gly, 1.1; Ala, 1.1; Val, 0.9; Leu, 6.4; Phe, 0.9; NH_3, 4.6; Arg, 4.0. Enzyme hydrolysis: NH_3, 1.0; Arg, 4.0; *Asp, 0.0;* Thr, 1.8; Ser, 3.0; Glu, 1.1; Gly, 0.9; Ala, 0.9; Val, 0.8; Leu, 6.2; Phe, 0.9.

S_{5-27} was also purified as described above. The *K* value is 2.2. Amino acid analysis: Asp, 1.0; Thr, 2.0; Ser, 2.7; Glu, 3.0; Gly, 1.0; Ala, 1.2; Val, 1.1; Leu, 6.2; Phe, 1.0; NH_3, 3.7; Arg, 3.9. Enzyme hydrolysis: NH_3, 1.1; Arg, 3.9; Asp, 0.8; Thr, 1.9; Ser, 3.0; Glu, 0.9; Gly, 0.8; Val, 1.2; Leu, 6.5; Phe, 1.0.

The four tricosapeptides, while homogeneous on thin layers of silica gel (system C), were indistinguishable from each other with respect to R_f values (0.30). On thin layers of cellulose (system A), small differences were noted: S_{5-27}, 0.48; 9-Gln-S_{5-27}, 0.44; 15-Asn-S_{5-27}, 0.40; 9-Gln-15-Asn-S_{5-27}, 0.40. Attempted gel electrophoresis failed because of poor staining. Thin-layer electrophoresis on microcrystalline cellulose plates (20 cm in length) showed the compounds to be homogeneous. All four tricosapeptides moved toward the cathode with a relative mobility of 0.7, in a buffer of pH 3.5 (pyridine-AcOH-H_2O, 1:10:489; 400 V; 1.5 h). The migration of arginine is defined as unity. In a buffer of pH 6.4 (pyridine-AcOH-H_2O, 100:2.5:400), the following relative mobilities (M_{Arg}) were observed (400 V, 1 h): S_{5-27}, 0.38; 9-Gln-S_{5-27}, 0.72; 15-Asn-S_{5-27}, 0.70; 9-Gln-15-Asn-S_{5-27}, 0.81.

ORD and CD spectra were recorded at room temperature with a Cary Model 60 spectropolarimeter fitted with a Model 6001 CD attachment. Cylindrical fused quartz cells of 1, 5, and 10 mm path length were used. Ultra-pure grade guanidine hydrochloride (Schwarz-Mann) was used without further purification. Other chemicals used were reagent grade. All solutions were centrifuged. Their pH was determined on a Radiometer Model 4 pH meter, but was not adjusted to any specified value because previous experiments had demonstrated that the ORD-CD spectra were not dependent on either pH or peptide concentration over the range of values measured. The concentration of the solutions was calculated from the recovery of quantitative amino acid analyses. Mean residue ellipticity values (in deg cm^2/decimol) were not corrected for the refractive indexes of the solutions, but the method of correction used by Tanford et al.[25] was applied for the values of rotation measured in guanidinium chloride solution. The concentration of guanidinium chloride was determined from the refractive index of the solutions.[26] The reversibility of "denaturation" was shown by diluting a solution of high salt concentration and comparing the values of rotation with those observed in solutions prepared at the lower concentration of guanidinium chloride.

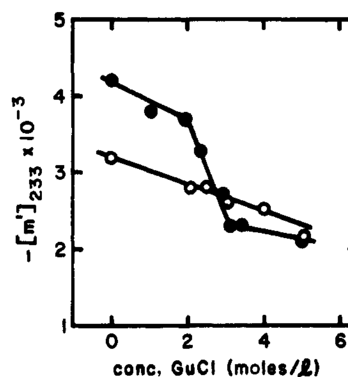

Figure 5. The effect of guanidinium chloride on the rotation (at 233 nm) of S_{5-27} (●) and 9-Gln-15-Asn-S_{5-27} (○).

Results

Optical rotatory dispersion and circular dichroism provide a simple method for studying the conformation of secretin. These spectra reveal a partially helical structure stabilized by folding of the chain.[7] Therefore, the ORD-CD spectra were also used in this study as evidence for folding. As shown in Figure 4, the CD spectrum of 9-Gln-15-Asn-S_{5-27} is markedly different from that of S_{5-27}. Thus replacement of both carboxyl groups with carboxamides led to a significant loss of "structure". In the CD spectrum of this analogue, the trough at about 222 nm is less pronounced than in the spectrum of S_{5-27}. The lower wavelength trough with a minimum of about 204 nm in S_{5-27} is shifted to 200 nm and increased in ellipticity in 9-Gln-15-Asn-S_{5-27}. The spectra of the monosubstituted analogues, 9-Gln-S_{5-27} and 15-Asn-S_{5-27}, are intermediate between those of S_{5-27} and the disubstituted analogue. We consider this as evidence for the involvement of both carboxyl groups in salt bridges. While the effect of replacing glutamic acid by glutamine could also be attributed to the difference in their conformational parameters,[27] this is considered unlikely because no dramatic enhancement of helical character was observed during stepwise chain lengthening when glutamic acid was incorporated into position 9, or even at the next step where this residue was not N-terminal.[28] Therefore we believe that the change caused by replacing glutamic acid by glutamine in S_{5-27} is due to the loss of an ion pair.

The finding that ion bridges play an important role in the stabilization of the conformation in secretin prompted experiments aimed at disrupting these salt bridges. It was somewhat surprising to note that, in 0.1 M NaOH, 6 M LiCl and 1.5 M NaCl, the ORD spectra were essentially the same as those observed in water. The emergence of a small trough at 209 nm was seen in 0.1-1.0 M HCl; KCl had no effect at low concentrations and caused turbidity at

a concentration of 2.7 M. Sodium chloride behaved similarly. The lack of any major effect of these electrolytes on the salt bridges could be explained by the assumption that electrostatic interaction is only one of the forces that contribute to a stable architecture, and that there is a degree of cooperativity[25] between the different forces. Supporting evidence for this assumption was found in the effect of guanidinium chloride on the ORD spectra of the tricosapeptides. This reagent is known to interfere not only with Coulombic interactions, but also with hydrogen bonding, and indirectly with hydrophobic interactions as well. As expected, at high concentrations, guanidinium chloride disrupted the secondary-tertiary structure of S_{5-27} (Figure 5). With increasing concentrations of the reagent, the decrease in rotation at 233 nm is at first moderate and gradual. A more abrupt change in the rotation takes place in solutions equal to and higher than about 2.5 M GuCl. The curve in Figure 5 demonstrates cooperativity between the various stabilizing forces; only a gradual drop in rotation was found in the case of 9-Gln-15-Asn-S_{5-27}. The other two analogues were not examined by this method because, as mentioned, the spectra of the two monosubstituted derivatives are intermediate between those of the unsubstituted and disubstituted tricosapeptides and the interpretation of changes in these intermediate spectra, in view of the inherent experimental error, seemed to be unwarranted.

Conclusions

The comparison of the CD spectra of aqueous solutions of S_{5-27}, 9-Gln-S_{5-27}, 15-Asn-S_{5-27}, and 9-Gln-15-Asn-S_{5-27} revealed that replacement of carboxyl groups by carboxamides significantly diminishes the helical character of secretin. Consequently, the assumption that ion pairs play an important role in the stabilization of the folded, partially helical structure is supported by these experiments. Obviously, in addition to ion pairs, other forces such as hydrogen bonding and hydrophobic interaction contribute at least equally to the formation of a well-defined architecture. This was shown by the observation that the spectra of the analogs of S_{5-27} still do not correspond to those of peptides with random conformations. Also, spectra taken in solutions of increasing guanidinium chloride concentration demonstrate cooperativity between the various stabilizing forces.

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References and Notes

(1) For paper V in this series cf. A. Bodanszky, M. A. Ondetti, and M. Bodanszky, *J. Am. Chem. Soc.*, **94**, 3600 (1972).
(2) This study is part of the Ph.D. dissertation of M. L. T. Fink, Case Western Reserve University, 1974.
(3) W. M. Bayliss and E. H. Starling, *J. Physiol (London)*, **28**, 325 (1902).
(4) J. E. Jorpes and V. Mutt, *Acta Chem. Scand.*, **15**, 1790 (1961).
(5) V. Mutt, E. J. Jorpes, and S. Magnusson, *Eur. J. Biochem.*, **5**, 513 (1970).
(6) M. Bodanszky and N. J. Williams, *J. Am. Chem. Soc.* **89**, 685 (1967); M. Bodanszky, M. A. Ondetti, S. D. Levine, and N. J. Williams, *ibid.*, **89**, 6753 (1967).
(7) A. Bodanszky, M. A. Ondetti, V. Mutt, and M. Bodanszky, *J. Am. Chem. Soc.*, **91**, 944 (1969).
(8) D. J. Patel, M. Bodanszky, and M. A. Ondetti, *Macromolecules*, **3**, 694 (1970).
(9) In the absence of high concentrations of electrolytes, secretin diffuses through membranes as a compact molecule (L. C. Craig, unpublished).
(10) Analysis of the sequence of secretin with the application of empirical conformational parameters (P. Y. Chou and G. D. Fasman, *Biochemistry*, **13**, 211, 222 (1974)) suggests that a helical stretch might be present in the C-terminal part of the chain. A similar prediction cannot be made for the N-terminal half of the molecule. A study of peptides with increasing length, corresponding to the sequence of 21-lysine secretin (K. W. Funk, Dissertation, Case Western Reserve University, 1974), also pointed to a helix near the C-terminus, stabilized (through folding) by residues in the N-terminal half. Contributions to the conformation of secretin by β-sheets and β-turns cannot be excluded in the Chou–Fasman analysis. The CD spectra allow the presence of some β-sheet-like structure, but it is difficult to visualize a small (about 10%) contribution in a chain of only 23 residues. Details of this analysis, including comparisons with the vasoactive intestinal peptide (VIP) and glucagon, have already been presented (M. L. Fink and M. Bodanszky, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **34**, 659 (1975) and will be published (M. Bodanszky, M. L. Fink, K. W. Funk, and S. I. Said, Proceedings of "Endocrinology 1975," supplement to Clinical Endocrinology, in press). More information on the architecture of secretin is being sought in this laboratory, through the study of synthetic analogs designed for this purpose.
(11) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959); M. Bodanszky, *Ann. N.Y. Acad. Sci.*, **88**, 655 (1960).
(12) M. Bodanszky, R. J. Bath, A. Chang, M. L. Fink, K. W. Funk, S. M. Greenwald, and Y. S. Klausner in "Chemistry and Biology of Peptides", J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 203.
(13) M. Bodanszky, *Nature (London)*, **175**, 685 (1955).
(14) W. König and R. Geiger, *Ber.*, **106**, 3626 (1973).
(15) M. Bodanszky, K. W. Funk, and M. L. Fink, *J. Org. Chem.*, **38**, 3565 (1973); M. Bodanszky, M. Kondo, C. Y. Lin, and G. F. Sigler, *ibid.*, **39**, 444 (1974).
(16) The hexacosapeptide S_{2-27} shows only marginal activity on the pancreas of the cat (personal communication from Professor Viktor Mutt, Medicinska Nobelinstitutet, Stockholm, Sweden). The tricosapeptides should be even less active or inactive and were therefore not assayed in this test. They are active, however, on different smooth muscle preparations. These experiments were carried out by Professor S. I. Said (Southwestern Medical School, The University of Texas, Dallas) and will be reported separately. An attempt to conclude the syntheses with the preparation of heptacosapeptides ran into technical difficulties. Since it would be interesting to observe the effect of the replacement of carboxyl groups on the typical activity of secretin, a new effort is planned for the preparation of these secretin analogues.
(17) S. Udenfriend, S. Stein, P. Bohlen, and W. Dairman in ref 12, p 655.
(18) R. H. Mazur, B. W. Ellis, and P. Cammarata, *J. Biol. Chem.*, **237**, 1619 (1962).
(19) T. Ziminski and E. Borowski, *J. Chromatogr.*, **23**, 480 (1966).
(20) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
(21) K. Hofmann, F. M. Finn, M. Limetti, J. Montibeller, and G. Zanetti, *J. Am. Chem. Soc.*, **88**, 3633 (1966).
(22) M. Bodanszky and M. A. Ondetti, *Chem. Ind. (London)*, 26 (1966).
(23) R. Willstätter and E. Waldschmidt-Leitz, *Ber. Dtsch. Chem. Ges.*, **54**, 113 (1921); H. Wieland, *ibid.*, **45**, 484 (1912); cf. also K. Kuromizu and J. Meienhofer, *J. Am. Chem. Soc.*, **96**, 4978 (1974).
(24) J. E. Jorpes, V. Mutt, S. Magnusson, and B. B. Steele, *Biochem. Biophys. Res. Commun.*, **9**, 275 (1962).
(25) C. Tanford, R. H. Pain, and N. S. Otchin, *J. Mol. Biol.*, **15**, 489 (1966).
(26) W. W. Kielly and W. F. Harrington, *Biochim. Biophys. Acta*, **41**, 401 (1960).
(27) P. Y. Chou and G. D. Fasman, *Biochemistry*, **13**, 211, 222 (1974).
(28) K. W. Funk, Ph.D. Dissertation, Case Western Reserve University, 1974.