

# A $^1\text{H}$ -NMR study of the solution conformation of secretin

## Resonance assignment and secondary structure

Angela M. Gronenborn, Gunter Bovermann and G. Marius Clore

*Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, FRG*

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The solution conformation of the 27 residue polypeptide hormone secretin has been investigated by  $^1\text{H}$ -NMR spectroscopy under conditions where it adopts a fully ordered structure as judged by circular dichroism spectroscopy, namely in an aqueous solution of 40% (v/v) trifluoroethanol. Using a combination of two-dimensional NMR techniques the  $^1\text{H}$ -NMR spectrum of secretin is completely assigned and its secondary structure is determined from a qualitative interpretation of the nuclear Overhauser enhancement data. It is shown that under these conditions secretin adopts a conformation consisting of an N-terminal irregular strand (residues 1–6) followed by two helices (residues 7–13 and 17–25) connected by a 'half-turn' (residues 14–16); the last two residues (26 and 27) are again irregular. This conformation is shown to be very similar to that of glucagon in perdeuterated dodecylphosphocholine micelles and to that of the active 1–29 fragment of growth hormone releasing factor in 30% (v/v) trifluoroethanol.

Secretin; Solution conformation; NMR; Nuclear Overhauser effect

### 1. INTRODUCTION

Secretin was the first gut hormone to be discovered when Bayliss and Starling [1] postulated the existence of a blood borne messenger that mediated the secretion of alkaline pancreatic juices following the perfusion of hydrochloric acid into denervated small intestine. Despite this early discovery it is only relatively recently that its physiological role in man as a major regulator of pancreatic exocrine secretion has

been established [2,3]. In addition to its action in physiological doses secretin exhibits a wide variety of pharmacological activities [3]. These include the inhibition of gastric acid secretion and gastrin release, the stimulation of insulin release and the inhibition of growth hormone release factor-stimulated growth hormone release.

Secretin is composed of 27 residues [4] and is a member of the glucagon superfamily [5] which also includes vasoactive intestinal peptide, gastric inhibitory peptide, the intestinal peptide PHI and GHRF. Of these hormones, the conformations of glucagon [6] and the active 1–29 fragment of GHRF [7] have been investigated by NMR spectroscopy under conditions where they adopt fully ordered structures, the former in perdeuterated dodecylphosphocholine micelles and the latter in 30% (v/v) TFE. Both these hormones were found to have a short N-terminal strand-like region followed by two helices (residues 10–14 and 17–29 in glucagon, and residues 6–13 and 16–29 in GHRF(1–29) $\text{NH}_2$  connected by a 'half-turn'. In

Correspondence address: A.M. Gronenborn, Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, FRG

**Abbreviations:** TFE, trifluoroethanol; GHRF, growth hormone releasing factor; NOE, nuclear Overhauser enhancement; NOESY, two-dimensional NOE spectroscopy; DQF-COSY, two-dimensional double quantum filtered homonuclear correlated spectroscopy; HOHAHA, two-dimensional homonuclear Hartmann-Hahn spectroscopy

neither case does the polypeptide chain fold back onto itself to form a globular tertiary structure. Secretin exhibits 52 and 22% amino acid sequence homology to glucagon and GHRF(1-29)NH<sub>2</sub>, respectively (fig.1). It is therefore of interest to examine the conformation of secretin under conditions where it adopts a fully ordered structure in order to determine whether sequence homology is related to structural homology in non-globular polypeptides.

To date the solution conformation of secretin has principally been investigated by CD spectroscopy [8-10]. In water, secretin appears to possess a very small amount of residual helicity although it is mainly disordered [8,10]. Ordered conformations, however, can be induced by solvents which reduce water activity such as 2-chloroethanol [9] and by anionic detergents and lipids [10].

In this paper we present a <sup>1</sup>H-NMR study on the conformation of secretin in 40% (v/v) TFE, a condition ensuring the complete conversion of the polypeptide into a fully ordered structure as monitored by CD spectroscopy. First, the <sup>1</sup>H-NMR spectrum is completely assigned by means of two-dimensional NMR techniques, and then the secondary structure elements are delineated from a qualitative interpretation of the NOE data.

## 2. EXPERIMENTAL

Secretin was a gift from Dr W. Koenig (Hoechst AG, FRG) and was >99% pure as judged by HPLC.

Samples for CD spectroscopy contained 0.1 mM secretin in 5 mM sodium phosphate buffer (pH 6.5) and varying amounts of TFE. CD spectra were recorded from 270 nm to 180 nm on a Jobin-Yvon Dichrographe Mark IV (Instruments SA, Paris) spectropolarimeter, connected to a PDB11/23 minicomputer for signal averaging and processing, at sensitivities in the range of 1-5 mdeg/cm with an instrumental time constant of 1 s. Cells with a



Fig.1. Comparison of the amino acid sequence of secretin with that of glucagon and GHRH(1-29)NH<sub>2</sub>.

1 mm pathlength were used and the spectra are the averages of 10 scans.  $\theta_R$  represent molar ellipticity values (deg·cm<sup>2</sup>·dmol<sup>-1</sup>) per mole of peptide residue, calculated from a mean residue weight of 113.2.

Two samples were prepared for NMR containing 5.6 mM secretin in 30 mM sodium phosphate buffer, pH 6.5, 40% (v/v) d<sub>3</sub>-TFE and either 60% D<sub>2</sub>O or 54% H<sub>2</sub>O/6% D<sub>2</sub>O. All NMR experiments were recorded at 20°C on a Bruker AM 500 spectrometer.

All two-dimensional NMR spectra were recorded in the pure phase absorption mode using the time proportional phase incrementation method [11]. The following spectra were recorded on both samples: NOESY spectra [12] at mixing times of 100 and 200 ms, and MLEV17 HOHAHA spectra [13] at mixing times of 18, 36 and 64 ms. In the case of the 54% H<sub>2</sub>O sample, the water resonance was suppressed by irradiation during the relaxation delay and in the case of the NOESY spectra during the mixing time as well. An additional NOESY spectrum with a mixing time of 200 ms was recorded on the 54% H<sub>2</sub>O sample in which the water resonance was suppressed by replacing the last 90° pulse in the sequence by a 90<sub>x</sub>- $\tau$ -90<sub>-x</sub> jump return pulse [14].

## 3. RESULTS AND DISCUSSION

Fig.2 shows the far ultraviolet CD spectrum of secretin in an aqueous solution (5 mM phosphate buffer at pH 6.5) containing varying amounts of TFE at 20°C. Qualitatively, the spectrum in the absence of TFE shows little ordered structure (<10% helix) in agreement with previous data [10]. Upon increasing the TFE concentration there is a significant increase in helical content as evidenced by the appearance of the distinctive high intensity negative band at 222 nm. This is easily seen from the variation of  $\theta_{222}$  as a function of TFE concentration (inset fig.2). The transition itself is essentially complete at a fairly low (~35%) concentration of TFE, indicating that only a small reduction in the water activity is required to induce a helical structure in secretin, consistent with the site of action of secretin at a membrane bound receptor. Further, the maximum value of  $\theta_{222}$  obtained in TFE is -27000 deg·cm<sup>2</sup>·dmol<sup>-1</sup> which is comparable, although slightly higher, to that ob-

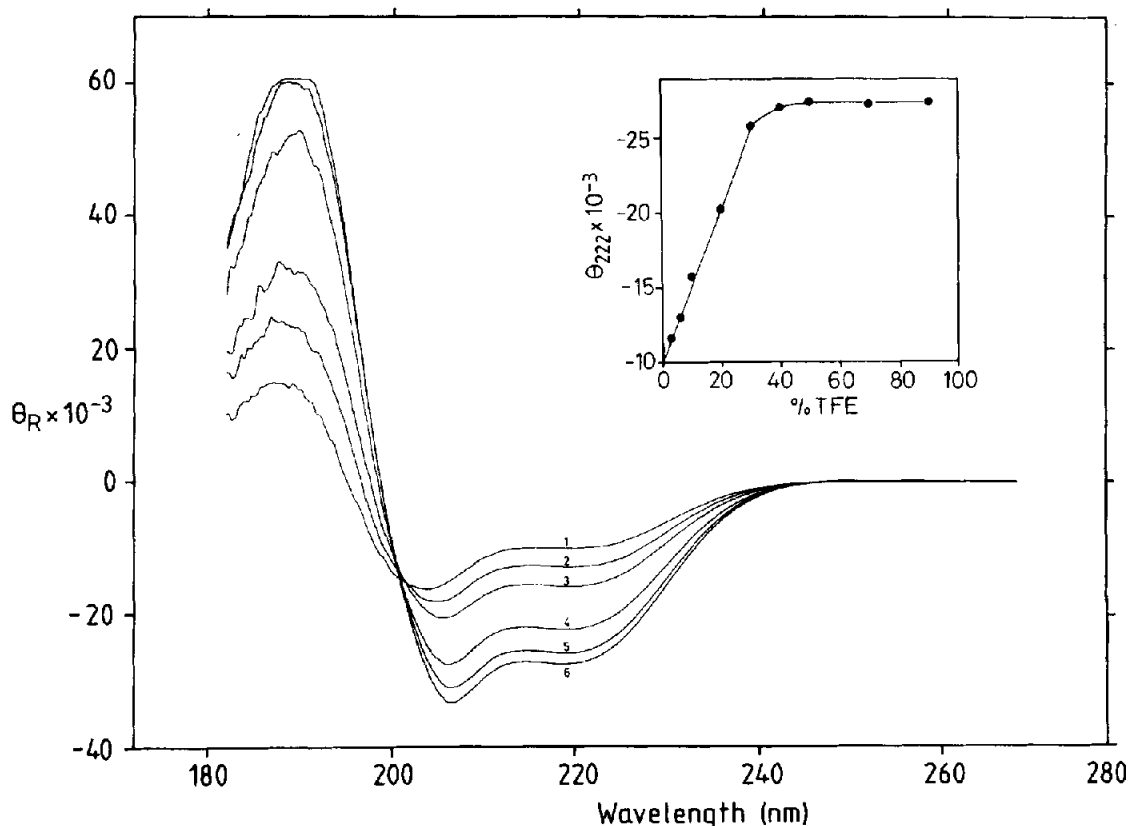


Fig.2. Far ultraviolet CD spectra of secretin as a function of TFE: 0% (1), 6% (2), 10% (3), 20% (4), 30% (5) and 50% (6). The inset shows the variation of  $\theta_{222}$  as a function of TFE concentration.

tained in the presence of sodium dodecylsulphate detergent micelles [10]. This corresponds to a helical content of 60–70% [15].

Sequence specific resonance assignments were made in a sequential manner [16,17]. The HOHAHA spectra, recorded at several mixing times in order to demonstrate successively direct, single and multiple relayed through-bond connectivities [18] were used to delineate complete spin systems. The NOESY spectra were used to demonstrate through-space ( $<5 \text{ \AA}$ ) connectivities in order to sequentially assign resonances via short range ( $|i-j| \leq 4$ ) NOEs involving the NH, C $^{\alpha}$ H and C $^{\beta}$ H protons, the most useful for this purpose being the NH( $i$ )-NH( $i+1$ ), C $^{\alpha}$ H( $i$ )-NH( $i+1$ ), C $^{\beta}$ H( $i$ )-NH( $i+1$ ) and C $^{\alpha}$ H( $i$ )-NH( $i+3$ ) connectivities. Examples of such spectra are shown in figs 3 and 4, and the complete list of assignments is given in table 1.

A summary of all the NOEs involving the NH, C $^{\alpha}$ H and C $^{\beta}$ H protons is given in fig.5. The information in this figure is sufficient to enable one to delineate secondary structure elements with confidence [17]. Unfortunately this information could not be supplemented by either NH exchange data or  $^3J_{\text{NH}\alpha}$  coupling constants. At pH 6.5, the exchange rates are too fast to be measured and data could not be collected at significantly lower pH values due to the known isomerisation of secretin at pH values below 5 involving a transpeptidation of the Ser-Asp peptide unit [19]. Although an  $\omega_1$ -scaled DQF spectrum [20] was recorded, it did not allow an estimate of  $^3J_{\text{HN}\alpha}$  coupling constants since the apparent peak-to-peak separation of the two antiphase components of the cross-peaks was between 7 and 9 Hz for all NH-C $^{\alpha}$ H cross peaks. In this case the linewidths of the NH resonances are 12–14 Hz so that the minimum peak-to-peak

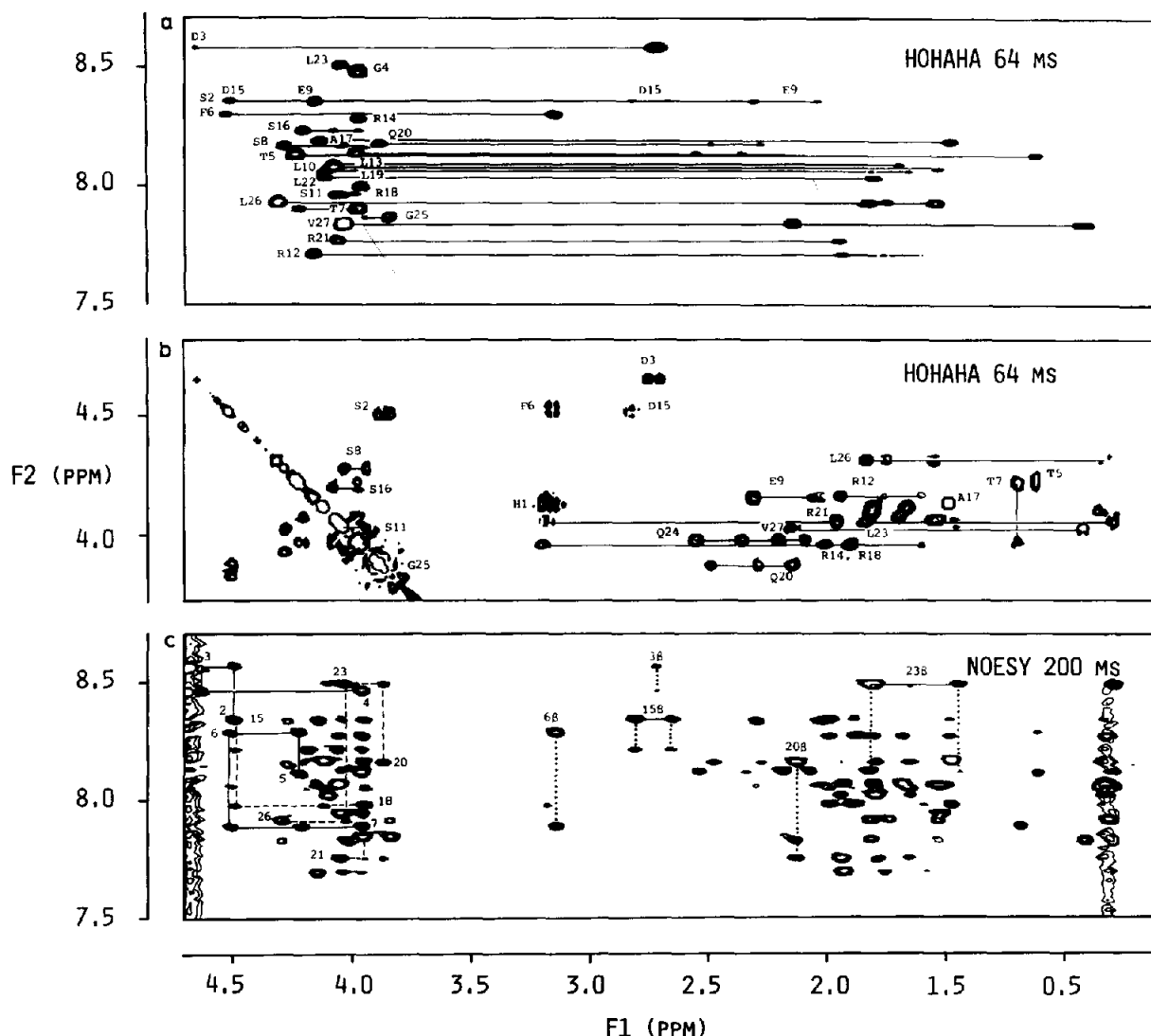


Fig.3. (a) HOHAHA spectrum of the NH(F2 axis)-aliphatic(F2 axis) region, (b) HOHAHA spectrum of the C $\alpha$ H(F2 axis)-aliphatic(F1 axis) region and (c) NOESY spectrum of the NH(F2 axis)-aliphatic(F1 axis) region of secretin in 40% TFE. The cross peaks in (a) and (b) arise from direct and multiple relay through bond connectivities. (The peaks labelled in (a) arise from direct NH-C $\alpha$ H connectivities.) The cross peaks in (c) arise from through-space (< 5 Å) connectivities and some examples of C $\alpha$ H(*i*)-NH(*i* + 1) (—), C $\alpha$ H(*i*)-NH(*i* + 3) (---) and C $\beta$ H(*i*)-NH(*i* + 1) (···) connectivities are shown with the labelling at the position of the intraresidue C $\alpha$ H-NH and C $\beta$ H-NH cross peaks. (a) and (c) were recorded on the 54% H<sub>2</sub>O/6% D<sub>2</sub>O sample while (b) was recorded on the 60% D<sub>2</sub>O sample.

separation ( $0.58 \Delta\nu_{1/2}$  [21]) of the antiphase cross peaks is 7–8 Hz.

The data in fig.5 reveal two distinct helical regions extending from residues 7 to 13 and 17 to 25 characterized by a continuous stretch of NH(*i*)-NH(*i* + 1) NOEs and the presence of C $\alpha$ H(*i*)-

NH(*i* + 3) and C $\alpha$ H(*i*)-C $\beta$ H(*i* + 3) NOEs. These helices are not completely regular as there are some gaps in the NH(*i*)-NH(*i* + 1) connectivities near or at the beginning of the helices (viz. from residues 7 to 9 and 18 to 19), although the C $\alpha$ H(*i*)-NH(*i* + 3) and C $\alpha$ H(*i*)-C $\beta$ H(*i* + 3) NOEs are present. The

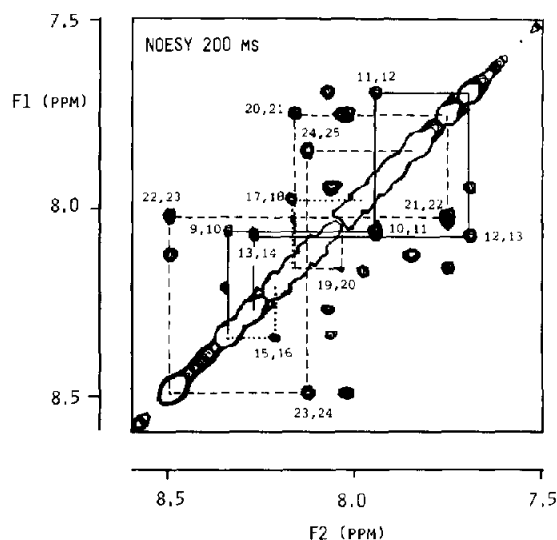


Fig.4. NH(F1 axis)-NH(F2 axis) of the NOESY spectrum of secretin in 40% (v/v) TFE, 54% H<sub>2</sub>O and 6% D<sub>2</sub>O, displaying through-space (<5 Å) connectivities. Symbols: NH(*i*)-NH(*i*+1) connectivities from residues 9-14 (—), 15-16 (···), 17-18 (···) and 19-25 (---).

region from residue 2 to 6 has the characteristics of an extended type strand typified by a continuous stretch of strong NH(*i*)-C<sup>α</sup>H(*i*+1) NOEs and the absence of other short range NOEs involving the NH and C<sup>α</sup>H protons. The linker region comprising residues 14 to 16 connecting the two helices has a pattern of short range NOEs typical of a half-turn [22]. Finally, no NOEs between protons separated by more than four residues in the sequence could be detected. Consequently, secretin

Table 1  
Proton resonance assignment of secretin in 40% (v/v) TFE

| Residue | NH   | C <sup>α</sup> H | C <sup>β</sup> H | Others                                                                 |
|---------|------|------------------|------------------|------------------------------------------------------------------------|
| His-1   | —    | 4.12             | 3.18,3.12        | C <sup>γ</sup> H 8.06 C <sup>δ</sup> 2H 7.11                           |
| Ser-1   | 8.35 | 4.59             | 3.87,3.81        |                                                                        |
| Asp-3   | 8.56 | 4.62             | 2.75,2.68        |                                                                        |
| Gly-4   | 8.47 | 3.96,3.96        |                  |                                                                        |
| Thr-5   | 8.12 | 4.20             | 4.23             | C <sup>γ</sup> H <sub>3</sub> 1.10                                     |
| Phe-6   | 8.29 | 4.51             | 3.14,3.14        | C <sup>γ</sup> H 7.19 C <sup>δ</sup> H 7.28 C <sup>ε</sup> H 7.19      |
| Thr-7   | 7.89 | 3.96             | 4.21             | C <sup>γ</sup> H <sub>3</sub> 1.19                                     |
| Ser-8   | 8.16 | 4.26             | 4.11,3.92        |                                                                        |
| Glu-9   | 8.34 | 4.13             | 2.30,2.02        | C <sup>γ</sup> H 2.30,2.30                                             |
| Leu-10  | 8.07 | 4.03             | 1.52,1.51        | C <sup>γ</sup> H 1.51 C <sup>δ</sup> H <sub>3</sub> 0.77,0.77          |
| Ser-11  | 7.95 | 4.04             | 3.96,3.96        |                                                                        |
| Arg-12  | 7.71 | 4.14             | 1.82,1.54        | C <sup>γ</sup> H 1.75 C <sup>δ</sup> H 3.16 N <sup>ε</sup> H 7.35      |
| Leu-13  | 8.08 | 4.05             | 1.79,1.68        | C <sup>γ</sup> H 1.68 C <sup>δ</sup> H <sub>3</sub> 0.82,0.82          |
| Arg-14  | 8.27 | 3.95             | 1.99,1.88        | C <sup>γ</sup> H 1.48 C <sup>δ</sup> H 3.14                            |
| Asp-15  | 8.35 | 4.59             | 2.80,2.65        |                                                                        |
| Ser-16  | 8.02 | 4.19             | 4.07,3.95        |                                                                        |
| Ala-17  | 8.17 | 4.12             | 1.47             |                                                                        |
| Arg-18  | 7.99 | 3.94             | 1.99,1.88        | C <sup>γ</sup> H 1.58 C <sup>δ</sup> H 3.17                            |
| Leu-19  | 8.09 | 4.10             | 1.78,1.64        | C <sup>γ</sup> H 1.64 C <sup>δ</sup> H <sub>3</sub> 0.85,0.85          |
| Gln-20  | 8.16 | 3.86             | 2.14,2.12        | C <sup>γ</sup> H 2.47,2.29 N <sup>ε</sup> H <sub>2</sub> 7.02,6.45     |
| Arg-21  | 7.77 | 4.04             | 1.94,1.94        | C <sup>δ</sup> H 1.77,1.65 C <sup>ε</sup> H 3.17 N <sup>ε</sup> H 7.19 |
| Leu-22  | 8.13 | 4.09             | 1.78,1.78        | C <sup>γ</sup> H 1.78 C <sup>δ</sup> H <sub>3</sub> 0.82               |
| Leu-23  | 8.50 | 4.19             | 1.80,1.43        | C <sup>γ</sup> H 1.43 C <sup>δ</sup> H <sub>3</sub> 0.77,0.77          |
| Gln-24  | 8.12 | 3.96             | 2.18,2.07        | C <sup>γ</sup> H 2.54,2.34 N <sup>ε</sup> H <sub>2</sub> 7.18,6.53     |
| Gly-25  | 7.86 | 3.95,3.83        |                  |                                                                        |
| Leu-26  | 7.92 | 4.29             | 1.82,1.53        | C <sup>γ</sup> H 1.73 C <sup>δ</sup> H <sub>3</sub> 0.82,0.82          |
| Val-27  | 7.83 | 4.12             | 2.13             | C <sup>γ</sup> H <sub>3</sub> 0.92,0.92 NH <sub>2</sub> 7.18,6.84      |

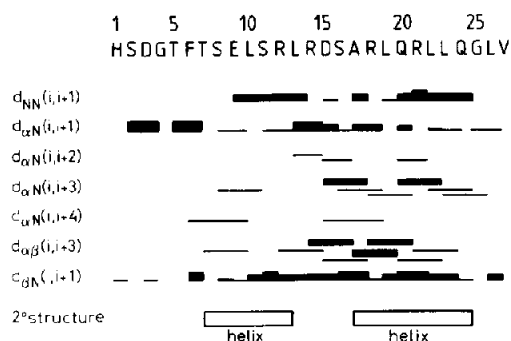


Fig.5. Summary of the NOE connectivities involving the NH, C $\alpha$ H and C $\beta$ H protons together with the secondary structure deduced from them. The NOEs are classified into strong, medium and weak according to the thickness of the lines.

does not fold back on itself into a tertiary structure.

A comparison of the secondary structure of secretin in 40% TFE with those of glucagon in perdeuterated dodecylphosphocholine micelles [6] and GHRF(1–29)NH<sub>2</sub> in 30% TFE [7], reveals a remarkable similarity in both the length and disposition of the secondary structure elements (see table 2) in accord with their sequence homologies. Considering the helices, the first helix of secretin (7 residues) has three residues in common with glucagon and two with GHRF(1–29)NH<sub>2</sub> while the second helix (9 residues) has two residues in common with glucagon and three with GHRF(1–29)NH<sub>2</sub>. In the case of the linker region (residues 14–16), secretin has two residues in common with glucagon and one with GHRF(1–29)NH<sub>2</sub>. The most extensive homology, however, between secretin and the two other hormones is at the N-terminal strand. Thus, seven of the first eight residues are identical in secretin and glucagon, and the one difference (Asp-3 in secretin for Glu-3 in glucagon) is conservative in nature. Likewise, three of the first seven residues are identical in secretin and GHRF(1–29)NH<sub>2</sub>. Thus, even in non-globular polypeptides, there appears to be a clear link between sequence and structural homology. This suggests that the other polypeptide hormones of the glucagon superfamily such as vasoactive intestinal peptide, gastric intestinal peptide and PHI(1–27) may also have similar conformations to secretin, glucagon and GHRF(1–29)NH<sub>2</sub>.

Table 2

Comparison of the secondary structure of secretin, glucagon and GHRF(1–29) derived from NOE measurements

| Secondary structure | Residues              |                       |                         |
|---------------------|-----------------------|-----------------------|-------------------------|
|                     | Secretin <sup>a</sup> | Glucagon <sup>b</sup> | GHRF(1–29) <sup>c</sup> |
| Irregular strand    | 2–6                   | 5–10                  | 1–5                     |
| Helix               | 7–13                  | 10–13                 | 6–13                    |
| Half turn           | 14–16                 | 14–16                 | 14–16                   |
| Helix               | 17–25                 | 17–29                 | 17–29                   |
| Irregular strand    | 26–27                 |                       |                         |

<sup>a</sup> This work

<sup>b</sup> [6]

<sup>c</sup> [7]

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