# NMR Spectroscopic Evidence That Helodermin, unlike Other Members of the Secretin/VIP Family of Peptides, Is Substantially Structured in Water

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ABSTRACT: The structure in water and additionally in 50% trifluoroethanol (TFE) solution of helodermin, an amidated peptide consisting of 35 amino acids, was elucidated by 2D $^1$H NMR spectroscopy initially from Hα chemical shifts and qualitative NOE data. Detailed structures were calculated from the quantitative NOE data which were used as distance restraints in molecular dynamics and energy minimization calculations. Regions of stable secondary structure were defined from the resulting final peptide conformations using a new fitting program that takes into account the summed RMS differences between all structures for short segments of 2−5 residues in length. This procedure allows a reasonably objective method of defining the edges of stable structure. In contrast to other members of the secretin/VIP family of peptides, helodermin shows a defined secondary structure in water alone and possesses an α-helix from Glu-9 to Leu-23 that was further stabilized and slightly extended (Phe-6 to Ala-24) on addition of TFE. The N- and C-termini were unstructured in both solutions. Such features, in particular the observation of a linear helix 18 ± 2 residues in length, are common to other members of the family and become more pronounced in hydrophobic environments. The data provide further circumstantial evidence that an α-helix conformation is necessary for receptor binding. The prolonged physiological action of helodermin, compared to its C-terminal deletion analogues and VIP, is at least in part due to the unusual stable secondary structure.

The north american lizard *Heloderma suspectum* (also known as Gila monster) is one of two members belonging to the species *helodermatidae*, which are nowadays the only living poisonous lizards. In 1982, it was observed that Gila monster venom exerts a secretory effect on guinea pig pancreatic acini and that it also increases the intracellular cAMP[1] level significantly. It was shown that the venom is able to inhibit $^{125}$I-vasoactive intestinal peptide binding to its membrane-bound receptor on pancreatic acini, which suggested the venom contains a factor that resembles the peptide hormones VIP and secretin and simultaneously stimulates adenylate cyclase activity and amylase secretion (Raufman et al., 1982). Subsequently, it has been shown that, in contrast to a simultaneous action, a 17.5 kDa (molecluar weight according to gel electrophoresis) peptide, PSF, is reponsible for the secretory action (Dehaye et al., 1983), while a 5.9 kDa peptide, helodermin, evokes cAMP production (Robberecht et al., 1983; Vandermeers et al., 1983).

Helodermin is an amidated peptide showing high sequence homology to members of the secretin/VIP hormone family (Figure 1) (Hoshino et al., 1984; Vandermeers et al., 1987) and is the first reported compound of this class that is not of mammalian or avarian origin. Compared toVIP, helodermin possesses a lower vasodilating activity upon canine femoral artery blood flow (Naruse et al., 1986), while both peptides are equally potent in enhancing prolactin secretion in anesthesized rats (Koshiyama et al., 1987). Helodermin has a more pronounced effect than VIP in stimulating thyroid hormone release in thyroid C cells (Grunditz et al., 1989), and both helodermin and PACAP suppress electrically evoked contractions of rat vas deferens, where VIP is without effect (Grundemar & Håkanson, 1992). Some cancer cell lines contain VIP receptors that show higher affinity toward helodermin than toward VIP. These receptors have consequently been named helodermin-preferring receptors (Robberecht et al., 1988). In addition, helodermin interacts with receptors specific to other members of the secretin/VIP family, e.g., with secretin receptors (Gillet et al., 1983). It has been widely observed that helodermin, compared to VIP, shows a prolonged effect in many cell systems [e.g., Naruse et al. (1986) and Grunditz et al. (1989)], and it has been suggested that this is caused by its C-terminus, which may protect the compound from enzymatic degradation but may also contribute to the receptor binding as proposed from deletion studies (Christophe et al., 1993; Li et al., 1993). As a consequence it was suggested that helodermin may prove superior to VIP as a means of treatment of asthma (Cardell et al., 1993).

Shortly after the characterization of helodermin, experiments with antibodies demonstrated the presence of helo-

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[1] Abbreviations: cAMP, cyclic adenosine monophospate; CD, circular dichroism; COSY, correlation spectroscopy; NOE, nuclear Overhauser enhancement; NOESY, nuclear Overhauser and exchange spectroscopy; PACAP, pituitary adenylate cyclase activating polypeptide; PRP, PACAP-related peptide; PSF, pancreatic secretory factor; PTH, parathyroid hormone; TFE, 2,2,2-trifluoroethanol; TOCSY, total correlation spectroscopy; VIP, vasoactive intestinal peptide.

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| *Peptide* | *Sequence§* |
|---|---|
| Secretin | H-S-D-G-T-F-T-S-E-L-S-R-L-R-E-G-A-R-L-Q-R-L-L-Q-G-L-V* |
| Glucagon | H-S-Q-G-T-F-T-S-D-Y-S-K-Y-L-D-S-R-R-A-Q-D-F-V-Q-W-L-M-N-T |
| PACAP-38 | H-S-D-G-I-F-T-D-S-Y-S-R-Y-R-K-Q-M-A-V-K-K-Y-L-A-A-V-L-G-K-R-Y-K-Q-R-V-K-N-K* |
| VIP | H-S-D-A-V-F-T-D-N-Y-T-R-L-R-K-Q-M-A-V-K-K-Y-L-N-S-I-L-N* |
| Helodermin | H-S-D-A-I-F-T-E-E-Y-S-K-L-L-A-K-L-A-L-Q-K-Y-L-A-S-I-L-G-S-R-T-S-P-P-P* |
| *Identity of helodermin with* | |
| Secretin | + + + + + + + + + + + + + |
| Glucagon | + + + + + + + + + + + |
| PACAP-38 | + + + + + + + + + + + + + + + + + + + + + + + + + + |
| VIP | + + + + + + + + + + + + + + + + + + + + + + + |

Footnote:

§ All the sequences are of the human peptides, apart from helodermin which is from *Heloderma suspectum*

* Amidated peptide

FIGURE 1: Representative sequences of peptides from the secretin/VIP family.

dermin-like immunoreactive material in various tissues of some mammals, e.g., brain, gut, and salivary glands of the rat (Robberecht et al., 1985a) as well as in the salivary glands and saliva of dog and man. As Gila monster venom is secreted by a gland in the mouth cavity, an overlap of exocrine and endocrine function for the peptide may exist; as yet, however, nothing is known about a biological role of helodermin in the lizard (Robberecht et al., 1985b; Christophe et al., 1993). Helodermin-like compounds have also been detected in thyroid C cells, the site of calcitonin production (Sundler et al., 1988). As helodermin is able to suppress calcium incorporation into bone, it is possible that helodermin-like compounds are, together with PTH, vitamin D, and calcitonin, involved in the control of calcium homeostasis (Grunditz et al., 1989), by enhancing the direct effects of PTH (Pfeilschifter et al., 1990). Moreover, it is possible that peptides related to helodermin are involved in the regulation of local pulmonary blood flow (Cardell et al., 1993). Unfortunately, sufficient material of mammalian origin for carrying out sequence analysis has not yet been isolated, so that the exact relation to lizard helodermin remains unknown.

In general, an understanding on a molecular level of how such small molecules as helodermin evoke their physiological action is of particular interest, as this could not only provide insight into the underlying biochemical mechanisms such as signal transduction but also provide an approach to the design of active agents, e.g., for the suppression or enhancement of their physiological actions. Ideally, one would like to initially elucidate both the structure of the hormone and its receptors and then continue by examining the structure, dynamics, and energetics of the hormone−receptor interaction.

X-ray structural analysis of members of the secretin/VIP family is problematic as such small peptides usually fail to crystallize, thus making NMR spectroscopy the method of choice for structural studies. Investigation of the receptors, on the other hand, is not yet possible, due to the unavailabilty of material and their high molecular weight which makes NMR studies impracticable (50 500 and 67 500 for VIP receptors in the liver; Rodríguez-Heche et al., 1994). Hence, one is restricted to the structure elucidation of the hormones themselves, and one must assume that a comparison of the structures of the different molecules, combined with results of the physiological effects of deletions and substitutions, reveals common or specific structural features that allow details of the possible mode of action of this class of compounds to be evaluated.

As the solution structures of some secretin/VIP family members have been determined in recent years (Braun et al., 1983; Clore et al., 1986, 1988; Gronenborn et al., 1987; Fry et al., 1989; Wray et al., 1993) by means of NMR spectroscopy, the structure of helodermin should provide further insight into the action and binding of this class of molecule. Here we present evidence that helodermin already has a stable secondary structure in water alone that is further stabilized in an hydrophobic environment. Crucially, helodermin has a greater preference for a central extended α-helix than other members of the family.

## MATERIALS AND METHODS

*Peptide Synthesis.* Helodermin was efficiently synthesized as the C-terminal amide by the Fmoc strategy on a Shimadzu simultaneous solid-phase peptide synthesizer PSSM-8 (Kyoto, Japan) as described previously (Nokihara et al., 1992). The cleaved peptide was purified by a single step of RP-HPLC, and its homogeneity was confirmed by liquid secondary ion mass spectrometry, amino acid analysis, and sequencing.

*CD and NMR Spectroscopy.* CD spectra were recorded at ambient temperature on a Jasco J-600 spectropolarimeter (Jasco, Tokyo, Japan) as described previously (Wray et al., 1993). Samples of the peptide (5 mg) for NMR measurements were dissolved in 540 μL of millipore water and 60 μL of $D_2O$ at pH 3.0 or in 50% TFE-$d_2$ by volume to give a final volume of 600 μL at pH 2.65 [TFE-$d_2$ was produced by fractional distillation of a 1:1 mixture of $H_2O$ and TFE-$d_3$ (Merck, Darmstadt, Germany)]. Spectra were recorded at 300 K on either a Bruker DMX 600 (sample without TFE) or a Bruker AM 600 (sample with TFE) NMR spectrometer without spinning using a triple-resonance probe head with a gradient unit or a dedicated 5 mm proton probe head, respectively. The $^1$H spectra were referenced to sodium 4,4-dimethyl-4-silapentane-1-sulfonate and indirectly to the residual water signal at 4.80 ppm, respectively. 2D phase-sensitive $^1$H COSY, TOCSY (mixing times 70 ms), and NOESY (mixing times 150 ms) spectra were recorded and processed as described previously (Wray et al., 1993).

*Structure Calculations.* The volumes of the integrated cross peaks from the NOESY spectra were determined using

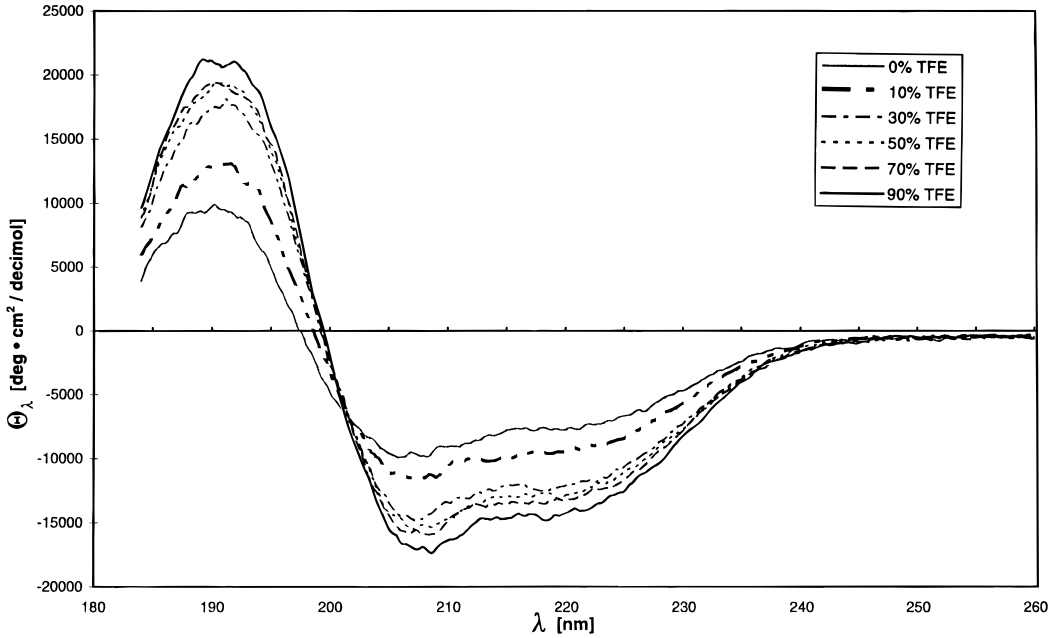


FIGURE 2: CD spectra of helodermin in aqueous solution containing TFE.

the AURELIA program (Neidig & Kalbitzer, 1990) and calibrated against those of the averaged Gln side-chain and terminal amide protons (0.19 nm). After correction for pseudoatoms, where appropriate, these values were used directly as distance restraints in molecular dynamics calculations using the program X-PLOR version 3.1 (Brünger 1992) on a DEC VAX 7000/16 workstation cluster (Digital Equipment Corp., Maynard, MA). An initial structure was calculated using randomized backbone dihedral angles and a short MD calculation at 300 K. The following simulated annealing calculation (Nilges et al., 1991), using floating stereospecific assignment (Weber et al., 1988), incorporated the following stages. After a short energy minimization, the first stage started with scaling of the weights of the NOE, constrained improper dihedrals, and nonbonded terms from initially small values to more realistic ones using in total 15 000 time steps each of 0.005 ps at 2000 K. The second stage performed a slow cooling of the system to 100 K in 38 stages each with ca. 150 time steps of 0.005 ps. The final stage consisted of 800 cycles of energy minimization. Floating stereospecific assignment was used because of the difficulties in achieving a priori stereospecific assignments. The resulting structures were displayed on a Hewlett Packard Apollo graphic workstation (Hewlett Packhard Co., Palo Alto, CA) using the program BRAGI (Version 5.0; Schomburg & Reichelt, 1988). Structure fitting criteria were derived either using modified programs from X-PLOR (see below) or computed within BRAGI using the dihedral angle order parameter defined previously (Hyberts et al., 1992).

## RESULTS

*CD Studies.* CD spectroscopy has been used here to give preliminary information on the secondary structure of helodermin in water and to monitor the response to the addition of TFE with the aim of finding conditions in which the peptide shows its most structured state. In sharp contrast to other small linear peptides of the secretin/VIP family such as PACAP (Wray et al., 1993) and PRP (Wray et al., 1995), helodermin in water alone shows a CD curve (Figure 2) with negative ellipticity at 206 and 220 nm and a positive band at ca. 190 nm indicative of significant amounts of stable α-helical structure. Addition of TFE causes these spectral features to become more pronounced up to 30% TFE and to remain relatively constant thereafter. Such a response to TFE is not uncommon and implies that helodermin adopts a limiting structured state in 30% TFE that shows little change on addition of further alcohol. Thus, qualitatively the CD spectra imply that helodermin contains regions of helical structure even without the addition of hydrophobic organic solvents. Hence we have used $^1$H NMR spectroscopy to define the positions of this secondary structure and to show the influence of TFE by determining the detailed molecular structure in both $H_2O$ and in 1:1 (v/v) TFE/ $H_2O$ in which the limiting structure is present.

*$^1$H NMR Spectroscopy.* Amino acid spin systems in both solutions were readily identified from 2D $^1$H phase-sensitive COSY and TOCSY spectra starting from the amide protons in the region 8.9−7.6 ppm and were confirmed by inspection of cross peaks in the high-field region corresponding to side-chain through-bond connectivities. Sequence-specific assignments then followed from sequential NOE signals in the NOESY spectra that reflect the short through-space distances between HN, Hα, and H$\beta$ of amino acid *i* and HN of amino acid *i*+1. These were significantly more easily identified in the 50% TFE solution, since the stabilized structure afforded stronger cross peaks, as can be seen for the HN−HN region of the NOESY spectra in Figure 3. Additional confirmation of the sequence assignments, particularly important for the water solution, were provided by medium-range NOEs in the regions of secondary structure where Hα−H$\beta$(*i*,*i*+3) and Hα−HN(*i*,*i*+3) NOEs were observed. Although the three terminal prolines could be identified in the TOCSY and NOESY spectra, these could not be unambiguously assigned in the water solution. In 50% TFE weak NOEs from Pro-33 to Ser-32 and Pro-34 and from Pro-34 to Pro-35 made an assignment possible. However, as there were no medium-range NOEs observed for these three C-terminal residues in either solution, this region of the

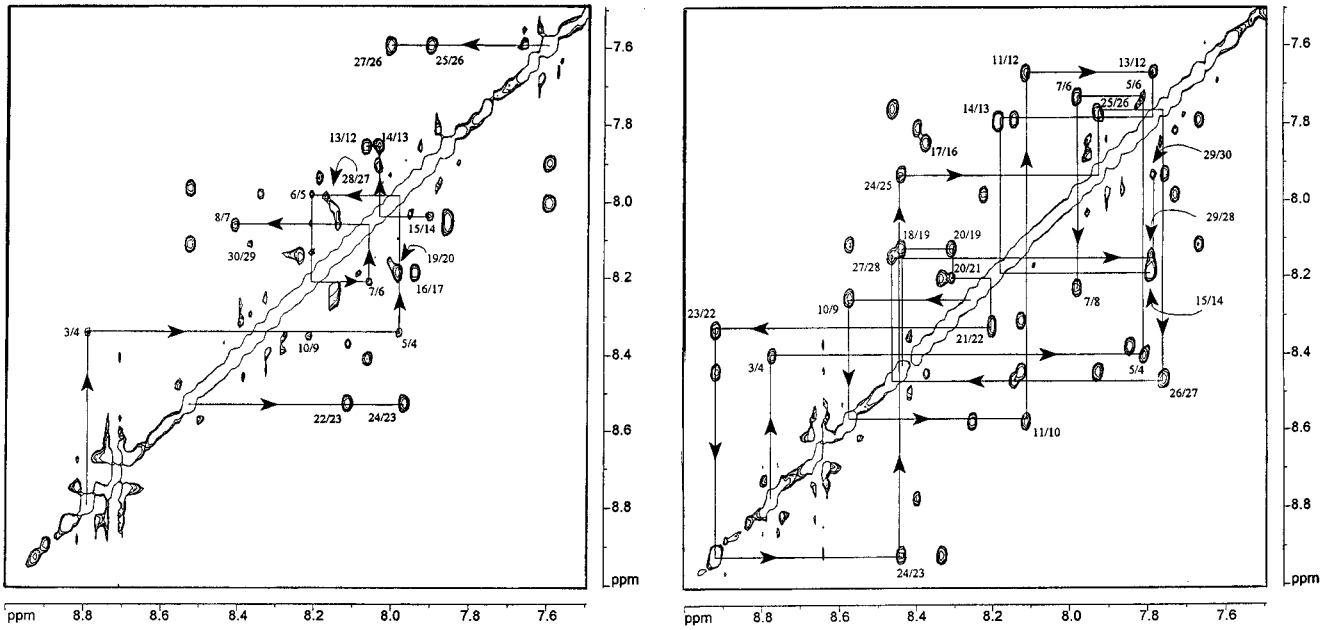


FIGURE 3: Amide region of the 2D NOESY spectrum (mixing time, 150 ms) of helodermin in water alone (left) and 50% TFE (right) showing sequential assignments.

molecule possesses no constraints and consequently behaves as though it was very flexible in the final calculated conformations.

Finally, the Hα's of Asp-3, Ser-32, Pro-33, and Pro-34 were hidden under the water signal in the water solution and were identified from spectra taken in $D_2O$, while Hα and H$\beta$ of Ser-11 in the TFE solution were accidentally equivalent. The data for the fully assigned peptide are available as Supporting Information in a table for both solutions.

*Secondary Structure from Hα Chemical Shifts and Qualitative NOEs.* The Hα chemical shift is known to be strongly dependent on the nature of protein secondary structure in both proteins and peptides. The Hα experiences an upfield shift relative to its random coil value in helices and a downfield shift in $\beta$-strands. Wishart et al. (1992) have shown that this provides a quick and reliable criterion for locating secondary structure. Hence helices are present when four adjacent residues show upfield shifts greater than 0.1 ppm, while downfield shifts of three adjacent residues greater than 0.1 ppm are indication of $\beta$-strand. Figure 4 shows the two situations found for helodermin. According to the above criterion, the peptide forms a helix between Tyr-10 and Ala-24 in water (Figure 4A) that is better defined in TFE solution (Figure 4B) and now extends from Phe-6 to Ile-26. This is apparent in the chemical shift changes produced on addition of TFE (Figure 4C) which show overall high field shifts in the region of the helix that are more pronounced at the helix termini. In both solutions residue 19, toward the center of the helix, shows a smaller shift difference than neighboring residues. In such a helical conformation Leu-19 comes into close proximity to Tyr-22 which can be expected to have a significant influence, through ring-current effects, on protons in its immediate vicinity. Hence the observation of a smaller shift difference for Leu-19 should not be interpreted as a flexible region between two helices.

Qualitatively similar conclusions regarding the position and differences in rigidity of the helix in the two solutions emerge from inspection of the medium-range NOE data (Figure 5). These also confirm there is no evidence of a discontinuity around Leu-19 in either solution. The absence of any long-range NOEs implies helodermin assumes a linear structure with a central helix and flexible termini.

*Final Structures and Structure Fitting.* In order to assess the quality and differences in the structure of helodermin in the two solutions, the quantitative NOE data were used as distance restraints in molecular dynamic/energy minimization calculations. A total of 199 and 325 distance restraints (from 63/90 intraresidue, 84/123 sequential and 52/112 other medium range NOEs distributed in the molecule as shown in Figure 6) for helodermin in water and TFE solutions, respectively, were used to calculate 100 conformations for each situation. Conformations for the fitting were chosen when there were no restraint violations greater than 0.01 nm and NOE energies below 4 and 15 kJ $mol^{-1}$, respectively. A total of 19 and 24 final conformations for the water and TFE solutions, respectively, satisfied these conditions and were used in the fitting procedure.

Normally in any fitting process two structures are compared by placing the midpoint of the region of interest together and optimizing until the smallest sum of the squared differences in atom positions is achieved. This is determined analytically in XPLOR using a matrix method (Kabsch 1976). In such a method the choice of the limits for the compared region of well defined structure is often subjective and inconclusive. One could use the limits defined by the Hα chemical shifts in order to define typical secondary structures or averaged values from DSSP algorithms. This has the disadvantage that regions of irregular but defined conformation may be overlooked. Alternatively, the limits can be varied and all structures compared with each other until there is a significant jump in the pairwise RMS deviation. Such a method is often inconclusive and still involves a number of subjective decisions.

Here we have attempted to develop a method that provides an objective criterion for finding optimal fitting regions and hence segments of defined conformation. In this new method the pairwise RMSDS program from X-PLOR was employed

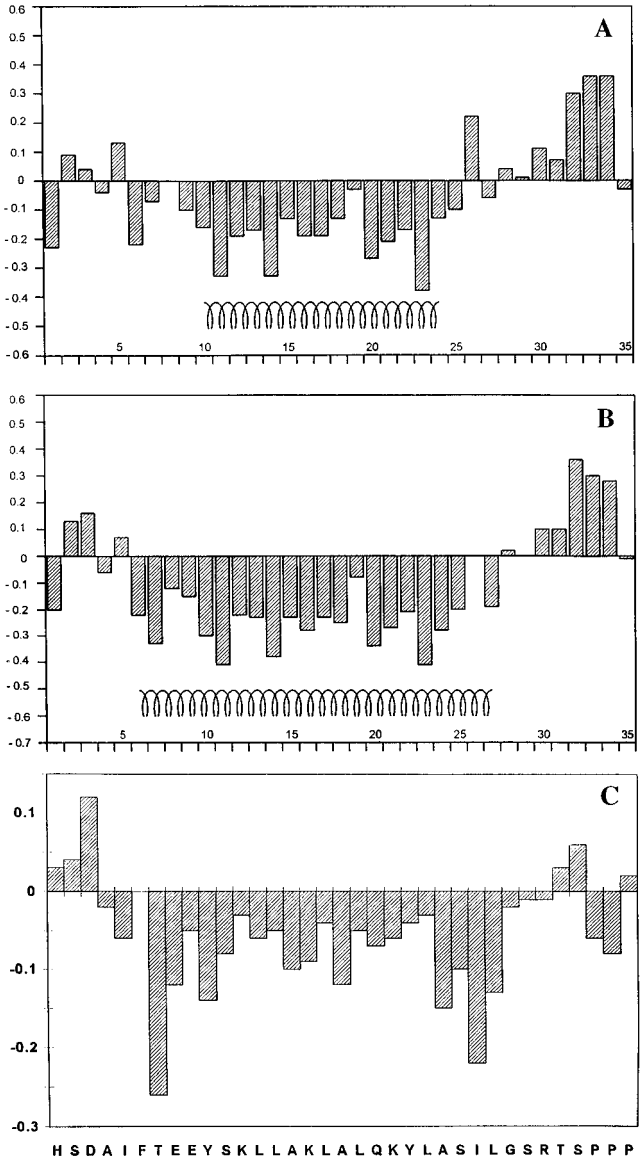


FIGURE 4: Chemical shift differences (ppm) of the α-protons between the experimental values and those for residues in a random coil for helodermin (A) in water alone, (B) in 50% TFE, and (C) their difference, B − A.

(Brünger X-PLOR 3.1 page 327), and short segments of two, three, four, and five residues in length were systematically compared pairwise for all selected final structures. For example, for an interval length of four residues the program started by comparing all structures for the segment His-1 to Ala-4, then Ser-2 to Ile-5, Asp-3 to Phe-6, and so on, until Ser-32 to Pro-35. The longest segment in the present situation was five amino acids in length as this corresponded to the longest NOE (*i*/*i*+4) observed in the sequence. The program calculated for each interval an average RMS differences (RMSD) for the backbone atoms from the pairwise comparison of all conformations. This value was then assigned to all residues in that interval. For a particular interval length, each residue was assigned a specific number of values depending on its position in the peptide, and the mean value was calculated. For instance for an interval of four, each residue in the center of the peptide was assigned four values as they appear in four different intervals, which were then averaged. This was repeated for each interval length, and the mean RMSD per backbone atom for each

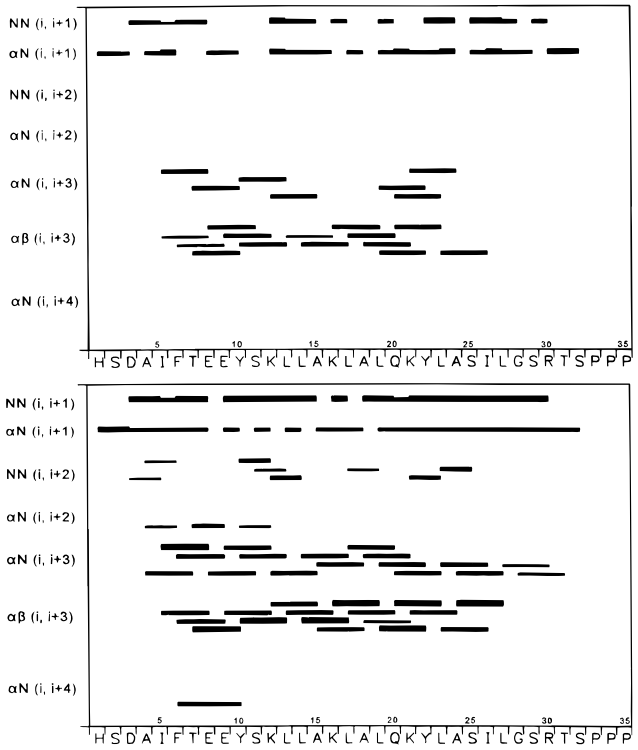


FIGURE 5: Summary of observed sequential and medium-range NOEs for helodermin in water alone (upper) and in 50% TFE (lower).

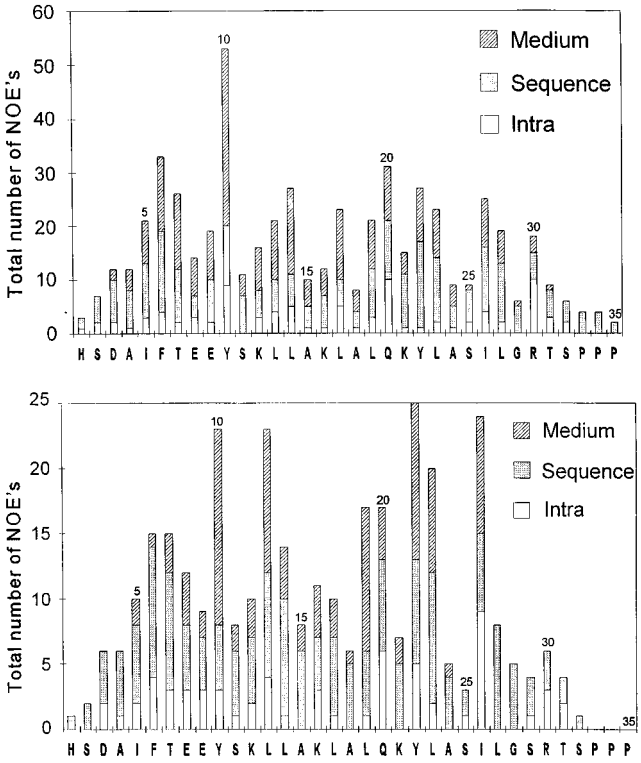


FIGURE 6: Distribution of the quantitative NOE's used in the retrained molecular dynamic calculations for helodermin in water alone (upper) and in 50% TFE (lower). Each NOE appears twice in this scheme as both residues, to which the interacting protons are attached, are shown.

residue was computed. These values were then plotted against the residue number, and the resulting plots for the two solutions are shown in Figure 7. An approach using a consecutive segment method has been described previously (Wagner et al., 1987; Fry et al., 1989); however, only one

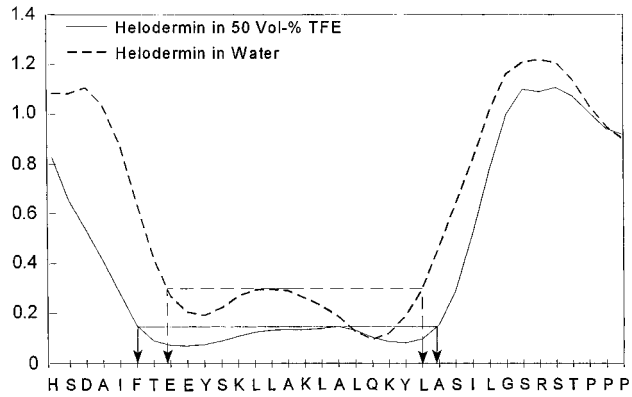


FIGURE 7: Mean RMS differences (Å) for backbone atoms in each residue, calculated as described in the text using a consecutive segment method, plotted against the residue number. The arrows, corresponding to the largest internal value, delineate the termini of secondary structural elements shown in Figure 8.

segment length was used, and a limited averaging process was used that was unable to cover the whole structure. More recently, an alterative method based on the calculation of backbone angular order parameters, incorporating the averaged 2D unit vectors with phases equal to the dihedral angle, has been used for structure comparisons (Hyberts et al., 1992). This offers an objective approach for assessing the interstructure variability, although the definition of the extent of particular secondary structures still requires a subjective assessment of two independent angular order parameters. This approach has also been implemented here, and a table of order parameters for the two relevant backbone dihedral angles is available in the Supporting Information.

In principle, the present function gives a relative measure of how well the backbone-atom positions in each amino acid in all final structures are defined. From Figure 7 it appears that the central section (residues 12−19) of the molecule, particularly in water, is more flexible than regions to right and left in keeping with DSSP analyses of individual structures. The termini are unstructured in agreement with the Hα chemical shift data. Comparison of the two curves (that are independent of the number of final structures used) confirms that helodermin adopts a better defined structure in 50% TFE. The backbone dihedral angle order parameters leads to identical conclusions.

For a meaningful fitting only amino acids with similar low RMS functions should be included. Hence we have chosen the maximum values at the center of the sequence as reference points and have used these to generate the fitting limits (arrowed regions in Figure 7). All structures were then fitted pairwise with all other structures (using RMS in X-PLOR) over the region Glu-9 to Leu-23 and Phe-6 to Ala-24 for the water and TFE solutions, respectively. In each case the conformation with the lowest mean RMS deviations was then chosen, and this acted as template for the fitting of all other structures to give the stereoview superpositions shown in Figure 8.

In summary, the Hα chemical shift data and the qualitative and quantitative NOE data provide convincing evidence that helodermin consists of a single helix in water with flexible N- and C-termini. Addition of TFE stabilizes the helix although the five N-terminal and eleven C-terminal residues remain unstructured.

## DISCUSSION

*Helodermin's Structure in Aqueous Solutions with and without TFE.* In most other studies of members of the secretin/VIP family of peptides (see the introduction) little evidence has been found for stable structure in water alone; only on exposure to a more hydrophobic environment, achieved by using aqueous organic solvents (such as TFE or methanol) or micelles, could secondary structure be detected by NMR spectroscopy. Helodermin is the first member to show stable structure without the necessity of using solvent mixtures. Significantly, the present results clearly show that addition of TFE causes stabilization of helical regions in the molecule, evidenced by the high field shift of Hα (Figure 4B) and lowered RMSD values (Figure 7), that already have a tendency for such structures in water alone. The residues undergoing the largest stabilization are those at the end of the helical region. There is no evidence of an induction of or change in secondary structure on adding TFE. Indeed, specific regions of the molecule, corresponding to the first five N-terminal residues and the last eight C-terminal residues, remain unstructured in both solutions. The absence of any long-range NOEs precludes the presence of any well-defined tertiary structure, and there is no evidence of a stabilized turn arising from interaction of the three terminal prolines and the helix, as proposed previously (Li et al., 1993). Under all conditions the molecule appears to be a linear helix, 16−19 residues in length, with unstructured termini.

We and others (Gronenborn et al., 1987) have pointed out the similarity of the structures of secretin, glucagon, GHRF-(1−29), PACAP38, PACAP27, and VIP under a variety of solvent conditions. As we predicted earlier (Wray et al., 1993) and now show experimentally, helodermin exhibits the same general features in having a disordered N-terminal region of 6−8 residues followed by an extended helix region of 18 ± 2 residues, which begins at or near the conserved residues Phe-6 or Thr-7. In contrast to the structures of VIP (Theriault et al., 1991) and PACAP (Wray et al., 1993), the helical region of helodermin shows no evidence of a central interruption and under both sets of solution conditions is significantly more stable.

The behavior of helodermin in solution has two consequences. The observation of the stabilization of preformed helical structure in helodermin on addition of TFE favors a similar rationale for other family members. Although stable structures are not usually easily observable by NMR spectroscopy in water alone, quantitative CD data imply a number of residues are involved in α-helices, for instance in PACAP (Wray et al., 1993). Such observations may now be rationalized by a transient involvement of a number of rapidly interconverting helix-containing species. These nascent helices are then stabilized in more hydrophobic environments and become readily observable by NMR techniques. An induction of such structures need not be invoked.

Secondly, the relationship of the solution structure of these conformationally mobile peptides to their receptor-bound structure is problematic. However, as an α-helical structure already exists almost complete (helodermin) or in part (other family members), it now appears most likely that they are bound in this conformation in the hydrophobic environment at or near to the membrane-bound receptor. It is worth

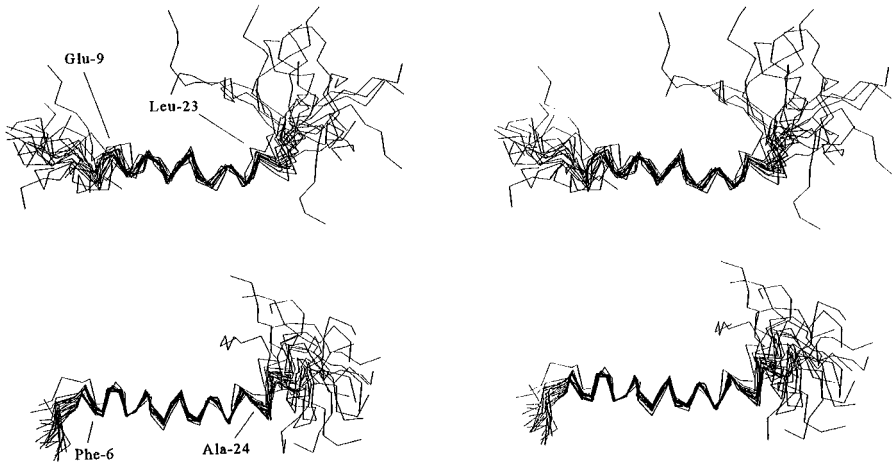


FIGURE 8: Stereoview superposition of all final restrained structures for helodermin, after alignment of the backbone atoms, in water alone (upper) and in 50% TFE (lower). The aligned regions were determined from Figure 7 and are from Glu-9 to Leu-23 and Phe-6 to Ala-24, respectively.

noting that for helodermin the initial section of the helix, from Phe-6 to Leu-17, is amiphipathic in character with a surface of hydrophobic residues (Phe-6, Tyr-10, Leu-13, Leu-14, and Leu-17) opposed by a surface of charged residues (Glu-8, Glu-9, Lys-12, and Lys-16). This feature is common to other family members and, as such, may suggest an orientation of the membrane-bound hormone. Such considerations could be taken as a starting point for site-directed point mutations in the molecule with a view to changing or enhancing their binding properties.

In summary, although currently the effect of the presence of the membrane-bound receptor on the structure of helodermin cannot be assessed, the presence of a quite well-defined conformation in water alone makes a completely new arrangement rather unlikely, especially as a more hydrophobic environment has a stabilizing, rather than destabilizing, influence. These conclusions serve as further justification for similar research that has been carried out on other members of the secretin/VIP family that interact with the same type of receptor which do not show stable secondary structure in pure water and where organic solvents had to be used to allow observation of the peptide's conformations.

*Structure and Function.* A considerable number of deletion and substitution experiments using different members of the VIP/secretin family have been used in an attempt to correlate amino acid sequence and the physiological effects of the peptides. Unfortunately, such studies on helodermin are rather rare and incomplete. This situation will undoubtedly change when both the primary structure of helodermin-like materials found in mammals and their biological role have been determined.

Most studies carried out on helodermin have dealt with the characteristic C-terminus which was quickly shown to be responsible for the prolongation effect that is typical for the peptide when compared with other family members (Naruse et al., 1986). A systematic study with helodermin analogues of canine vasoactivity and their binding to the helodermin-affinity receptor and influence on cAMP production in human neuroblastoma NB-OK-1 cells highlighted the importance of the C-terminal residues of helodermin for its activity and prolonged action compared to VIP and its C-terminal deletion analogues (Li et al., 1993). In particular there was a marked decrease in activity and loss in the prolongation effect on going from helodermin(1−31) to helodermin(1−30) and shorter analogues. The retained activity of the former analogues negates arguments involving proline protection against degradation by carboxypeptidases.

The behavior of the deletion peptides was then rationalized using secondary structure predictions made with the Chou−Fasman algorithm (Chou & Fasman, 1974), according to which the C-terminus of helodermin(1−31) and its longer analogues have a certain tendency for $\beta$-turn formation which is absent in the 1−30 variant. It was then concluded that the C-terminus stabilizes the active core of helodermin, possibly by giving protection against enzymatic digestion. This, however, cannot be confirmed with the results of our studies, as a shielding turn conformation has not been observed (Figure 8). The conformational stability of helodermin in water alone leads us, on the other hand, to an alternative explanation for the prolonged physiological effects. If a helical structure is required for receptor binding, as we suggest above, helodermin should be able to undergo a stronger interaction as the helix is already present in water alone and is noticeably more stable than in other members of the secretin/VIP family. It is possible that 31 amino acids present a critical value such that shorter segments no longer stabilize a central helix in pure water.

Experimental support for the importance of the stabilized central region of helodermin, compared to other members of the VIP/secretin family of peptides, is afforded by the behavior of chimeric VIP-helodermin peptides in the same NB-OK-1 cell system (Li et al., 1993). Thus VIP(1−11)-helodermin(12−31) has an affinity that is ca. 10 times that of VIP and comparable to helodermin, while shortening of the helodermin region by deletion of residues 12−14 in the chimeric peptide VIP(1−11)-helodermin(15−31) shows a considerable loss in binding affinity probably through a shortening and loss of helical structure.

The present structural study provides direct evidence that the central amino acid sequence of helodermin has a particularly high preference for helical secondary structure that appears to play a significant role in enhancing binding affinity to the presumably membrane-bound receptor. Clearly, further physiological studies of further peptide derivatives related to the helodermin sequence with a view to clarifying their influence on binding is justified.

## SUPPORTING INFORMATION AVAILABLE

Table of the $^1$H chemical shifts of helodermin in water at pH 3.0 and 300 K, and in 50% TFE at pH 2.65 and 300 K together with a table of the backbone dihedral angle order parameters (6 pages). Ordering information is given on any current masthead page.

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