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Conformational behavior of the linear hexapeptide senktide: a receptor specific tachykinin analog

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A receptor selective linear hexapeptide tachykinin analog, senktide, is shown to be highly ordered in solution. The conformational restriction is attributed to steric and electrostatic interactions produced by *N*-methylation of the third amino acid residue in the sequence and the negatively charged N-terminus. The structure of senktide is described as a dynamic mixture of similar conformations where the predominant one is a distorted antiparallel hydrogen bonded β-pleated sheet. The observed senktide-receptor specificity is suggested to result from a selection of this or a closely related conformation.

Receptor selective; Conformational restriction; NMR, 2D; CD

1. INTRODUCTION

Senktide is a linear hexapeptide which has the sequence succinyl-Asp-Phe-MePhe-Gly-Leu-Met NH_2. It is highly potent and selective to the NK_3 tachykinin receptor subtype [1] of which neurokinin B is the endogenous ligand. The tachykinins bind with varying affinities to the NK_1, NK_2, and NK_3 receptors and have a common C-terminal pentapeptide segment Phe-Xxx-Gly-Leu-MetNH_2 (Xxx = Phe, Tyr, Ile, Val) [2]. It has been suggested that this flexible segment controls receptor-ligand affinity and that one of the many allowable conformational states may be selected for or induced by a particular receptor. One explanation for the receptor specificity of synthetic tachykinin analogs is that they show restricted conformational behavior.

In recent years evidence has been provided which shows that small peptides occasionally have conformational order in solution. Here we show that, on the basis of the nuclear Overhauser effect cross peaks between backbone amide NH protons, senktide has an unusually high degree of conformational order in solution.

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2. EXPERIMENTAL AND RESULTS

The CD spectra of senktide in methanol and in trifluoroethanol, shown in fig.1, are similar in character. The two broad negative bands near 200 nm and 215 nm show that the peptide is folded in both solvents [3]. It has been pointed out that methanol is a relevant solvent for peptide studies, since it often induces the same conformations as found in phospholipid environments [4]. Senktide was characterized by mass spectrometry and the possibility of conformational order due to dehydrocyclization of the succinyl group (a cyclic structure) was excluded [5].

The proton NMR spectrum of senktide in methanol was assigned using homonuclear Hartmann-Hahn (HOHAHA) spectroscopy. In methanol solution senktide has major (80%) and minor (20%) components which give rise to similar patterns of magnetization transfer in HOHAHA spectroscopy. Sequential cross peaks, $C_\alpha H_{i-1}$–NH_i, resulting from through space interactions ob-

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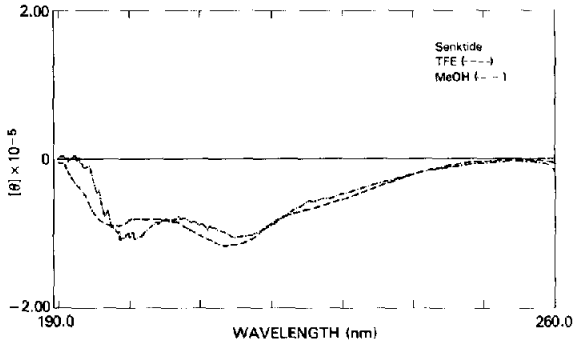

Fig.1. The circular dichroism spectrum of senktide in trifluoroethanol (---) and in methanol (·····) at 0.19 mM and 0.17 mM, respectively. Both spectra were taken with a 0.1 cm cell on a Jasco J-600 spectropolarimeter.

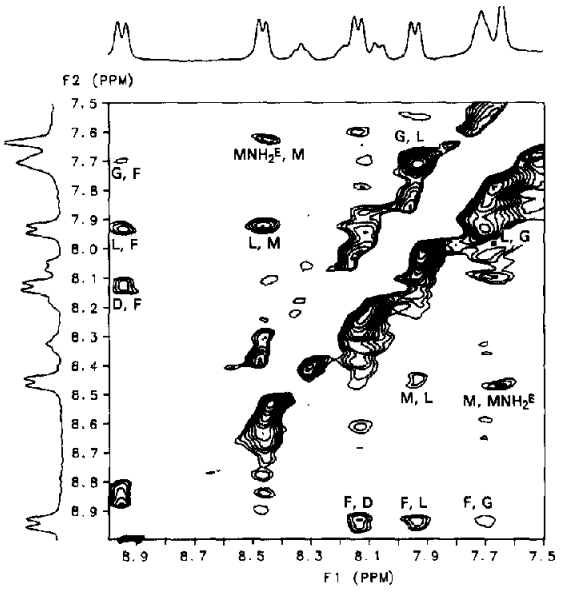

Fig.2. The amide proton region of the ROESY spectrum with a mixing time of 160 ms (the $T_{1\rho}$ values for the amide protons are 150–200 ms) on a 20 mM sample of senktide in methanol at 18°C. The 1D spectrum is plotted along both the F2 and F1 axes. The abundant peaks (80%) in the 1D spectrum are shown to be from the *trans*-configuration (Phe-MePhe-Gly) while the minor peaks (20%) are believed to be from the *cis*-configuration of the peptide. ROE cross peaks are labeled *i,j* and indicate the amide proton resonances of residue *i* along the F2 axis and residue *j* along the F1 axis. The carrier is positioned at 4.91 ppm and rf transmitter power is attenuated to give a 90 pulse width of 67 μs to ensure a Hartmann-Hahn mismatch and minimize indirect HOHAHA-ROESY magnetization transfer. The data were collected on a Varian XL-300 spectrometer with 1024 points in t_2 and 160 points in t_1 and were apodized with a Gaussian function and zero filled to 2048 points in both dimensions before Fourier transformation.

tained in rotating frame nuclear Overhauser effect (ROESY) [6,7] spectroscopy confirm a *trans*-conformation for the Phe-MePhe peptide bond of the major component. The minor component is assigned to the *cis*-conformation by observation of ROESY cross peaks between the $C_\alpha H_i$ and $C_\alpha H_{i+1}$ resonances of the Phe-MePhe residues. Because of low *S/N* ratios for the minor conformer cross peaks, we were only able to study the *trans*-isomer.

The ROESY spectra were collected with mixing times of 120 and 160 ms. At the longer mixing time, experiments were run with the carrier positioned at the $C_\alpha H$ and the NH regions of the spectrum to detect any cross peaks arising from indirect transfer of magnetization. The amide proton region of the ROESY spectrum, in fig.2, shows cross peaks between all of the neighboring amide proton resonances and also between the Phe N-H (residue 2) and Leu N-H (residue 5) amide proton resonances. This is the first report of nuclear Overhauser effect cross peaks between backbone amide NH protons (i.e., $d_{NN}(i,i+3)$) three residues apart. For the *S/N* ratios in our spectra, the cross peak intensities between amide protons correspond to internuclear distances of 2.7 ± 0.5 Å [8]. The results demonstrate that the peptide has a high degree of folding [9], a very unusual result for a short linear peptide in solution [10]. The short distance between the Phe and Leu amide protons shows the presence of long range order. The origin of this order may be due to intramolecular NH···OC hydrogen bonding. Also, one observes all of the interresidual NOE connectivities expected for an ordered peptide (fig.3). A possible structure which is consistent with the experimental results is similar to the antiparallel β-pleated sheet conformers proposed for cyclic hexapeptides [11]. Here (as shown in fig.4), the Phe carbonyl oxygen and Leu amide proton and the Leu carbonyl oxygen and the Phe amide proton are hydrogen bonded. A distortion of the β-pleated sheet conformation by formation of bifurcated hydrogen bonds, also shown in fig.3, allows the amide protons of Phe and Leu to be close together in space and thus give rise to the ROE cross peak observed. This conformation satisfies many of the other observed ROE cross peaks. Additional data which

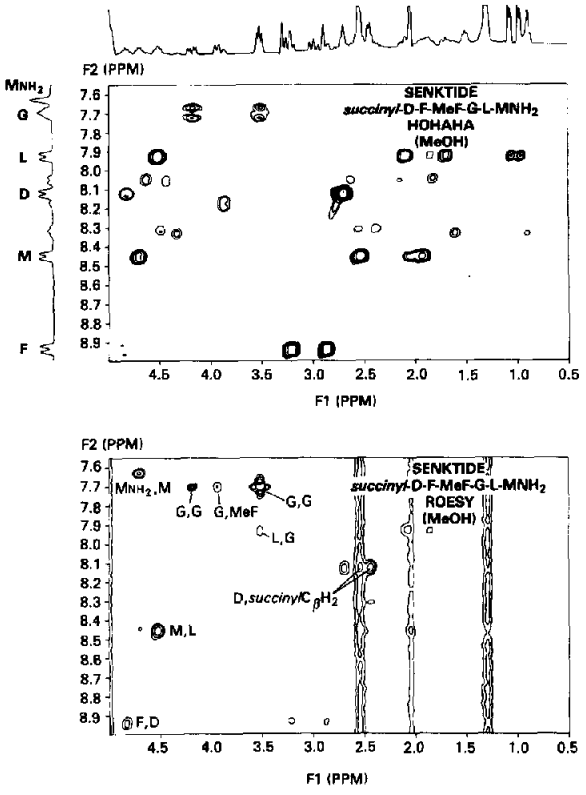

Fig.3. The amide-aliphatic region of the HOHAHA and ROESY spectra with a mixing time of 160 ms. The experimental details are given in the caption to fig.2.

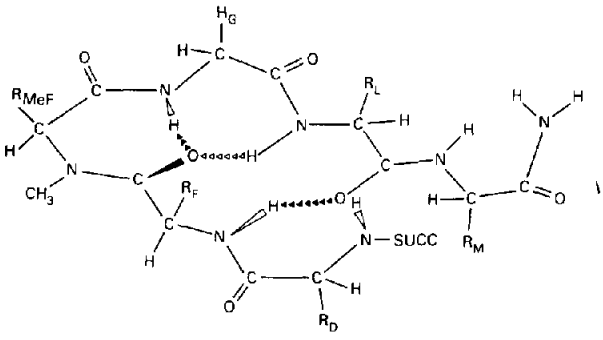

Fig.4. A distorted antiparallel hydrogen bonded (dashed lines) β-pleated sheet conformation proposed for senktide. The standard one letter notation for amino acids is used to label the individual residues.

support ordered structure come from the vicinal spin-spin coupling constants. For Asp and Phe, the $^3J_{NH-CH}$ values are 8.1 Hz and 9.1 Hz, respectively. For the MePhe residue, the $^3J_{CH-CH}$ values are 3.4 and 11.2 Hz. Furthermore, we were able to obtain a crystalline material from methanol solution. The X-ray structure of this peptide will be the subject of a separate report (Silverton, J., unpublished). Also detailed conformational energy calculations show that the distorted antiparallel β-pleated sheet conformation represents a favorable low energy structure (Sumner, S., Jaing, S.-P., Jernigan, R. and Ferretti, J., in preparation).

Chemical shift temperature coefficients show that the Leu and Gly amide protons are more solvent protected ($\leq$0.005 ppm/°C) than the other amide protons (0.01 ppm/°C). This suggests that the Phe to Leu (NH···OC) hydrogen bond is less favorable than the Leu to Phe (NH···OC) and the Gly to Phe (NH···OC) hydrogen bonds. To explain this behavior and also to account for all of the observed ROESY cross peaks, a set of conformational states in dynamic equilibrium is proposed. Here, the equilibrium consists of a set of conformations where the Phe-MePhe-Gly segments are similar in orientation while the succinyl-Asp and Met-NH_2 residues are more flexible. This allows for a set of conformational states where most of the peptide remains in a relatively fixed orientation. The observed CD spectrum (fig.1) is consistent with a mixture of folded conformational states. The structure shown in fig.3 represents one of the possible high population conformers, where all conformers are proposed to be structurally similar.

3. DISCUSSION

Although highly ordered structures for linear hexapeptides in solution are generally uncommon, senktide has restricted conformational behavior due to steric hindrance produced by *N*-methylation of Phe. This steric hindrance at the Phe-MePhe linkage results in repulsive interactions associated with the two Phe rings and the NCH_3 group. The conformational behavior of the NK_1 and NK_2 endogenous ligands and other receptor selective tachykinin analogs is much more flexible than senktide (Sumner, S. and Ferretti, J.A., unpublished). This particular type of conformational restriction found for senktide may result in the increased NK_3 receptor specificity. The receptor-ligand specificity of senktide may result from ac-

cessibility of a hydrophobic region created in the distorted antiparallel β-pleated sheet structure. The observation that the Phe-MePhe sequence is important for providing the repulsive interaction which leads to increased peptide order may be useful in future design of molecules.

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