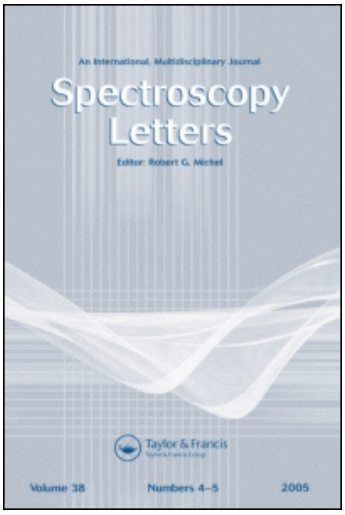

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CONFORMATIONAL STUDIES ON PENTAGASTRIN BY 2D NMR AND RESTRAINED MOLECULAR DYNAMICS

Key words: Conformation, 2D NMR, Pentagastrin, RMD

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ABSTRACT

The present study uses 2D NMR to assign all resonance lines of pentagastrin completely. From 2D NOESY and coupling constants $^3J_{HN\alpha}$, interproton distance and torsion angle Φ restraints were obtained. Based on them, the model of conformation for pentagastrin was proposed by restrained molecular dynamics calculation. It is shown that the C-terminal part of pentagastrin forms a γ turn with a weak H-bond between the CO of Met and the NH of Phe. A strong H-bond between indole NH of Trp and β-carboxyl group of Asp stablizes the folded form in DMSO, which may result in a significant increase in the population of a favorable conformation for interaction with receptor.

INTRODUCTION

Gastrin is a 17-amino acid peptide hormone which promotes the stomach's secretion of gastrin acid. Intensive studies of structure activity relationships have been carried out by synthetic analogues, CD spectra, NMR and molecular mechanics, and show that the C-terminal tetrapeptide of gastrin, Trp-Met-Asp-Phe-NH_2 is the minimum segment which has the physiological activity[1,2]. CD studies proposed that tetragastrin was an extended coil in H_2O, but in trifluoroethanol (TFE) or aqueous solution containing phospholipids adopted a defined, compact conformation in which the peptide backbone was folded and the aromatic side chains were <8 Å apart[3]. ^1H NMR study found that in tetragastrin and octagastrin, a hydrogen bond between Nle CO and Phe NH might be formed. Indole NH of Trp might also be involed in a hydrogen bond in TFE, but in DMSO they were in a random form[4], therefore, Feeney et al concluded that the tetrapeptide in DMSO was an extended coil with the Trp and Phe aromatic residues separted by at least 5 Å with no CO-NH intramolecular hydrogen bond[5]. Molecular mechanics calculation showed that indole NH of Trp, sulfur atom of Met had a close approximation of three dimensional array of binding sites to that of 5,1-benzothiazocine. It has thus been theoretically demonstrated that gastrin and 5,1-benzothiazocine could bind with an identical receptor[6,7]. In short, there is some disagreement in the literature concerning the structure of the C-terminal tetrapeptide of gastrin and up to date, no data of X-ray crystallographic analysis are available.

It is well known that 2D NMR techniques and restrained molecular dynamics have been used in determining the structure of proteins and polypeptides[8,9]. From the two-dimensional nuclear Overhauser enhancement spectra (NOESY) and coupling constants $^3J_{HN\alpha}$, approximate distance and dihedral angle restraints are derived and used as the basis for there-dimensional structure determination with a restrained molecular dynamics algorithm. In this paper, solution conformation of pentagastrin were studied in this way. It was found that pentagastrin was not in extended form in DMSO. C-terminal Met-Asp-Phe formed a γ turn with a weak H-bond between the CO of Met and the NH of Phe. A strong H-bond between indole NH of Trp and

β-carboxyl group of Asp stablized the folded form and might alter the populations of the allowed conformations in solution and result in a significant increase in the population of a conformation where the active groups were favorably arranged for interaction with receptor.

EXPERIMENT SECTION

Pentagastrin was purchased from Sigma Chemical Co. Its amino acid sequence is Boc-β-Ala-Trp-Met-Asp-Phe-NH_2. Sample was dissolved in DMSO-d_6 at a concentration of 20 mM.

All NMR spectra were run on Bruker AM500 equipped with Aspect 3000 computer at 300K. High resolution one-dimensional NMR spectrum was collected in 16K data blocks. All two-dimensional spectra (COSY[10], Relayed COSY[11], DQF-J resolved spectrum[12] and NOESY[13]) were recorded with the standard procedures and presented in the absolute value mode with symmetrization. Free induction decays were apodized by multiplication with sine bell function before Fourier tranformation. The solvent signal was supressed by continuous low power selective irradiation during relaxation delay. 90° pulse width was 5.5 μs. In all cases chemical shifts were reported in parts per million (ppm) from DMSO. The digital resolution of 5.6Hz/pt for COSY and 2.5Hz/pt for NOESY spectra were achieved by appropriate zero-filling in t_1 dimension only. NOESY spectra were obtained at mixing times 600 ms and 900 ms.

Φ angles were calculated by Karplus equation $^3J_{HN\alpha} = 6.4\cos^2\theta - 1.4\cos\theta + 1.9$, $^3J_{HN\alpha}$ were obtained from 1D NMR spectrum and DQF-J resolved spectrum, Φ is defined as: $\theta = |\Phi - 60^\circ|$. The maximum θ value on the positive side of the portion of the Karplus equation is 6.90 Hz at $\theta = 0^\circ$, therefore all coupling constants over this value give a unique solution to θ [14].

Restrained molecular dynamic calculations were carried out on 4D/20G graphics workstation using the program *Biograf*. Distance restraints of interproton were obtained by comparison with cross-peak intensities in two independently measured NOESY spectra. The distances were divided into three classes corresponding to

strong, medium and weak NOEs . For all $r<2.7$ Å, r_{ij} was set to 2.5 Å; For all r in the range 2.7 Å$<r<3.5$ Å, r_{ij} was set to 3.2 Å; For all r in the range 3.5 Å$<r<5.0$ Å, r_{ij} was set to 4.5 Å; For distances involving methyl groups, an additional correction of 0.5 Å is usually added[16] and interproton distances are calibrated during calculation. Vicinal spin-spin coupling constants can provide useful information supplementing the interproton distance restraints derived from NOE data, especially when the molecular is flexible in solution, fewer NOEs would be expected in a NOESY spectrum. According to $^3J_{HN\alpha}$, the angle Φ was obtained and restrained to the range (-160°, -80°) where $^3J_{HN\alpha}$ is >8.0 Hz[15,16].

RESULTS AND DISCUSSION

1. NMR studies:

The first step in the structural analysis of pentagastrin was to obtain a COSY spectrum shown in Fig. 1. As the spin system of Met is different from other residues and the amide proton of β-Ala shifts upfield evidently because of Boc-group, so their spin systems can easily be identified from COSY spectrum. The spin sysytem of Trp indole ring was also shown in Fig. 1, where the resonance at 10.98 ppm which couples with Trp 2H can be assigned to be indole ring NH. Fig. 2 shows DQF-J resolved spectrum of aromatic region, doublet of Phe2,6H, quarter of 3,5H, triplet of 4H and two protons of terminal amino can be assigned. Because of poor-resolved αH of spin system 1 and 2 (Fig. 1), their βH can only be resolved by NH-βH cross-peaks in Relayed COSY spectrum (Fig. 3). Spin systems 2 and 3 can be identified by intraresidual NOEs between Phe 2,6H and βH, Trp 2H and βH (Fig. 4). The rest belongs to Asp. Therefore all of the protons in the peptide were assigned, in which the protons of Phe ring and C-terminal amino were first identified correctly. The complete resonance assignments are given in Table 1.

2. Restrained molecular dynamics calculation on pentagastrin:

The distance restraints of interproton and Φ restraints are listed in Table 2. Restrained molecular dynamics calculation proceeded in two stages starting from extended coil using three different first-stage to explore the dependence of the final

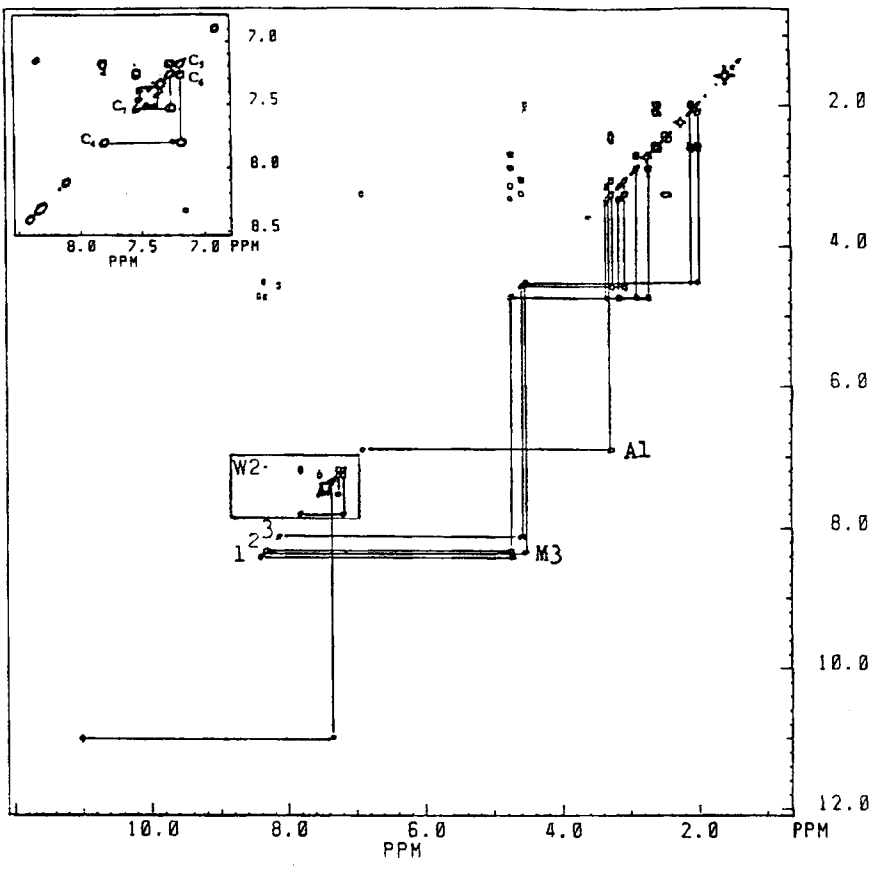

Fig. 1. Contour plot of 500 MHz COSY spectrum of pentagastrin in DMSO-d_6 at 300K.

structure on the way the restraints were introduced. Conformation I adopted progressively rolling method, i.e. restraints were first applied, then distance restraints were used progressively in turns of intraresidues, interresidues and long ranges. Conformation II, Φ restraints were first applied, then all distance restraints were used. Conformation III, all distance restraints were first applied, Φ restraints were then used. After each phase, energy minimization was performed to reduce the structural distortions that accompany the increase in kinetic temperature due to the

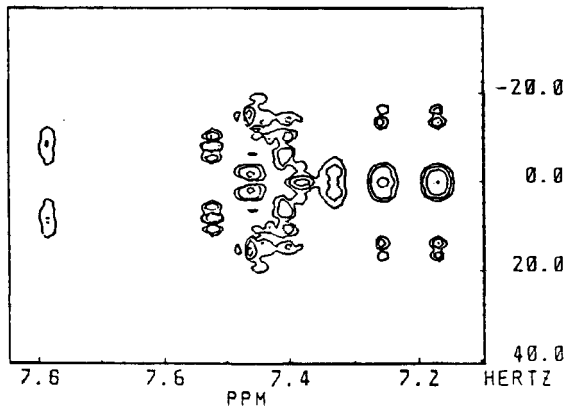

Fig. 2. Aromatic region of 500 Mhz DQF-J resolved spectrum of pentagastrin in DMSO-d_6 at 300K.

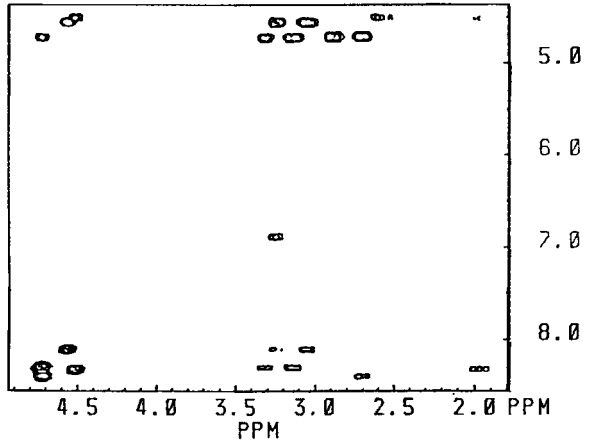

Fig. 3. Portions of Relayed COSY spectrum of Pentagastrin in DMSO-d_6 at 300K.

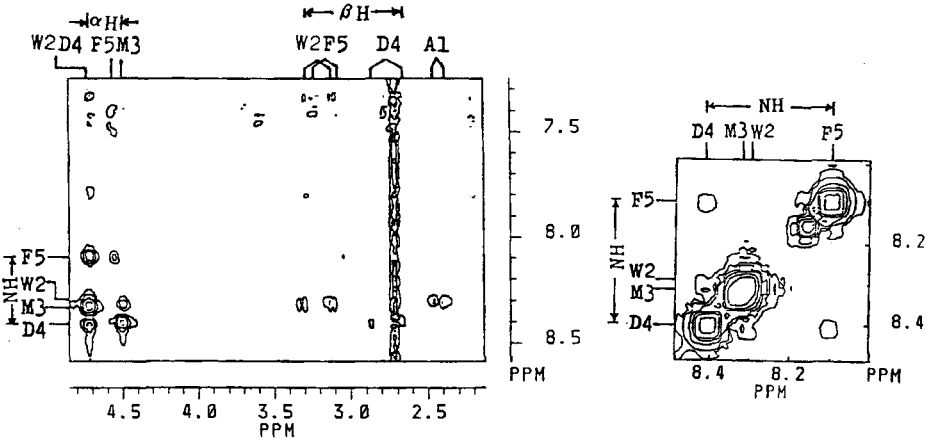

Fig. 4. Portions of NOESY spectrum of pentagastrin in DMSO-d_6 at 300K with mixing time of 900 ms.

TABLE 1

Chemical Shifts of The Assigned Resonances of Pentagastrin in DMSO

Residues	NH	αH	βH	others
Ala1	6.88	2.35, 2.39	3.23, 3.27	
Trp2	8.30	4.73	3.13, 3.31	2H: 7.33, 4H: 7.79, 5H: 7.17
				6H: 7.25, 7H: 7.52, NH: 10.98
Met3	8.32	4.51	1.97, 2.07	γH: 2.54, 2.58, ϵCH_3: 2.21
Asp4	8.40	4.70	2.70, 2.87	
Phe5	8.10	4.57	3.05, 3.22	2,6H: 7.42, 3,5H:7.41, 4H: 7.46
				cisNH: 7.38, transNH: 7.43

TABLE 2

Restraints for Molecular Dynamics Calculation of Pentagastrin

Residue	NOEs	Distance Restraints	$^3J_{HN\alpha}$	Φ Restraints
Ala1	Ala1 αH-Trp2 NH	3.2 Å		
Trp2	Trp2 αH-Trp2 NH	2.5 Å		
	Trp2 NH-Trp2 βH	3.2 Å		
	Trp2 7H-Phe5 αH	3.2 Å	7.7	-88°
Met3	Met3 αH-Met3 NH	2.5 Å		
	Met3 αH-Asp4 NH	2.5 Å		
	Met3 NH-Asp4 NH	3.2 Å		
	Met3 εC-Trp2 2H	5.0 Å		
	Met3 εC-Phe5 2H	5.0 Å	8.4	-93°
Asp4	Asp4 NH-Asp4 βH	4.5 Å		
	Asp4 αH-Asp4 NH	2.5 Å		
	Asp4 NH-Phe5 NH	3.2 Å	7.7	-88°
Phe5	Phe5 αH-Phe5 NH	2.5 Å		
	Phe5 NH-Phe5 βH	4.5 Å	8.4	-93°

large decrease in the restraints energy. The second stage, in which all structures were treated identically, comparised a 5 ps equilibrium and thermalization followed by a 10-ps restrained molecular dynamics at 300K. The temperature during this second stage remained constant at 300K, and the structure did not undergo large conformational changes; the final 5-ps were used for computing average structures. The equilibrium energy of three final conformations were 251.63, 260. 02 and 253.92 Kcal/mol respectively.

TABLE 3

The ψ and ω Values of Conformations I, II, III and Their Root-Mean-Square Differences (RMSD)

	ψ				ω			
Residues	I	II	III	RMSD	I	II	III	RMSD
Ala1	176.1	-175.6	179.8	3.4	-175.2	179.7	-175.4	2.3
Trp2	106.6	101.2	-66.1	80.2	165.5	171.3	-16.9	82.4
Met3	32.6	60.1	44.5	11.3	-178.6	151.7	-169.3	16.6
Asp4	27.1	-75.0	59.3	57.3	-149.1	-51.8	177.9	55.2
Average				38.0				39.2

3. Model building of solution conformation:

The RMS (root-mean-square) distance differences of backbone atoms and those involving the side chains for three conformations I, II and III were calculated which were found to be 0.272 ± 0.009 Å and 0.854 ± 0.091 Å. This result suggests that three conformations can converge to a single structure, especially the backbone of the three structures superimpose well. Dihedral angles ω and ψ for three conformations were calculated (Table 3). Except for individual residues, ψ and ω angles of most residues are near each other and their ω angles are close to ± 180. For ψ and ω, the dihedral angle RMS differences were found to be 38 and 39. Large deviation mainly occurred in Trp and Asp, for which the RMS difference of ψ and ω were 80, 57 and 82, 55. This is due to the lack of NOEs for the poorly defined regions, showing that side chains of the two residues have large mobility in solution.

A γ turn is defined by the existence of a hydrogen bond between CO group of one residue (i) and the NH of the (i+2) residue. The criterion used for hydrogen bonds

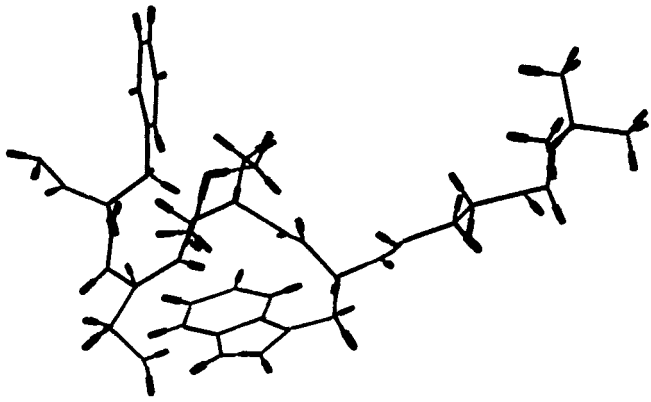

Fig. 5. Stereoview of the conformation I of pentagastrin obtained from restrained molecular dynamics calculation based on NOEs and $^3J_{HN\alpha}$.

is the electrostatic binding energy E[17,18], in which partial charges are placed at C, O ($+q_1$, $-q_1$) and N, H ($-q_2$, $+q_2$) atoms, $E=q_1q_2$ (1/r(ON)+1/r(CH)-1/r(OH)-1/r(CN))*f where $q_1=0.42$ e, $q_2=0.2$ e, e is the unit charge, r(AB), the interatomic distance from A to B in angstrom units, the dimensional factor f=332 and E is in Kcal/mol. A large negative E value signifies a strong bond, the cutoff value used for hydrogen bond definition by Kabsch & Sander is -0.5 Kcal/mol. In view of conformation I which has the lowest energy in three conformations (Fig. 5), C-terminal Met-Asp-Phe forms γ turn with a weak H-bond between the CO of Met and the NH of Phe (E=-0.64 Kcal/mol), this is consistent with the C-terminal part of CCK_4 (tetragastrin), CCK_5, CCK_6[19] and conformational energy calculations which suggested the possibility of a C_7 structure as the favoured conformation of the C-terminal tetrapeptide amide[20]. A number of γ turns were found to occur at ligand binding sites or active sites where they appear to well conserved during evolution[17]. Asp at i+1 position in γ turn was found frequently[17]. A strong H-bond between indole NH of Trp and β-carboxyl group of Asp (E=-1.39 Kcal/mol) might stablize the folded form or result in a significant increase in the population

of a favorable conformation where the active groups were arranged for interaction with receptor.

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