A CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDY OF OXYTOCIN AND ITS OLIGOPEPTIDES.[1]

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Summary: Fourier-transformed carbon-13 magnetic resonance spectra at 25.16MHz were obtained for oxytocin and various oligomeric intermediates in dimethylsulfoxide-d_6 and deuterium oxide. All resonances of the oligopeptides were assigned. The C-13 spectra were found to be sensitive to the presence of cis and trans peptide bonds in X-proline peptides.Changes in the resonances of amino acid residues in the cyclic portion of oxytocin were observed upon cyclization of the acyclic nonapeptide precursor. The data indicate no major conformational differences for the hormone in dimethyl sulfoxide and water.

A model for the conformation of oxytocin in deuterated dimethylsulfoxide (DMSO-d_6) has been derived primarily from proton magnetic resonance studies (1-3). The present investigation was undertaken in order to test the usefulness of carbon-13 magnetic resonance (CMR) in conformational studies of biologically active peptides. Hydrogen-bonding and changes in secondary structure should be detected by CMR because direct observation of the backbone carbons is possible.

MATERIALS AND METHODS

Oxytocin (40 mg/0.2 ml) and the following intermediates were examined in DMSO-d_6 (Z=benzyloxycarbonyl; Bzl=benzyl): Z-Pro-Leu-Gly-OEt, I; Z-Pro-Leu-Gly-NH_2, II; Z-Cys(Bzl)-Pro-Leu-Gly-NH_2, III; Z-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, IV; Z-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, V; Z-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, VI; Z-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, VII; Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, VIII. The assignments of I to VIII are given in Table 1. A number of deprotected peptides were studied in order to evaluate the influence of protecting groups, as well as to measure solvent effects (DMSO-d_6 and D_2O) on linear peptides:

[1] The present study has been presented in part at the 3rd American Peptide Symposium, Boston, June 1972. At the same symposium Dr. F.A. Bovey reported a related investigation.

Table 1 Chemical shifts and line assignments of the C-13 resonances of oxytocin and protected intermediates in DMSO-d_6.

Residue		I	II	III	IV	V	VI	VII	VIII	Oxyt.
Gly	αCH_2	42.53	43.52	43.60	43.60	43.60	43.60	43.72	43.60	43.62
	C=O	171.18	172.42	172.46	172.46	172.46	172.46	172.50	172.50*	
Leu	αCH	52.34	53.06	53.06	53.06	53.06	53.06	53.06	53.06	53.06
	βCH_2	42.33	41.73	41.73	41.81	41.81	42.01	41.93	41.93	41.93
	γCH	25.64	25.72	25.84	25.85	25.83	25.83	25.83	25.91	25.72
	δCH_3	24.44	24.52	24.52	24.52	24.52	24.52	24.52	24.52	24.64
	δCH_3	23.13	23.13	23.13	23.13	23.13	23.13	23.13	23.13	23.13
	C=O	174.09	173.77	173.65	173.65	173.69	173.65	173.65	173.89	173.77
Pro	αCH	61.47	61.61	61.49	61.49	61.61	61.61	61.61	61.47	61.69
		60.69	60.61							
	βCH_2	32.79	32.67	30.41	30.48	30.48	30.48	30.48	30.29	30.40
		31.47	31.48							
	γCH_2	25.23	25.52	26.03	26.03	26.03	26.03	26.03	25.91	26.03
	δCH_2	48.69	48.69	48.49	48.57	48.57	48.57	48.57	48.57	48.49
		48.17	48.17							
	C=O	173.29	173.09	173.13	173.13	173.17	173.1	173.13	173.01*	
Cys	αCH			54.13	52.27	52.34	52.36	52.46	52.27	54.93*
	βCH_2			37.36	37.24	37.24	37.24	37.36	37.24	41.61
	C=O			171.06	170.63	170.59	170.59	170.63	170.51	169.40
Asn	αCH				53.35	51.27	51.27	51.35	51.35	52.07
	βCH_2				39.03	38.63	38.63	38.63	38.75	38.53
	γC=O				172.85	173.01	172.85	173.01*	172.50*	
	C=O				172.85	173.01	172.85	172.81*	172.89*	
Gln	αCH					56.12	53.74	53.74	53.85	55.64*
	βCH_2					29.29	29.61	29.61	29.61	28.41
	γCH_2					33.07	32.99	33.07	33.07	33.19
	δC=O					175.52	175.56	175.76	175.60	175.76
	C=O					172.34	172.30	172.30	172.30	
Ile	αCH						60.89	58.50	58.67	60.61
	βCH						38.04	38.24	38.16	37.56
	γCH_2						26.03	26.03	25.91	26.01
	δCH_3						16.97	16.97	16.97	17.09
	δCH_3						12.53	12.60	12.60	12.79
	C=O						172.54	172.81	172.38*	
Tyr	αCH							57.90	55.52	56.24*
	βCH_2							38.24	38.16	37.36
	C_4							158.59	157.39	157.39
	C_2, C_6							131.83	131.76	131.64
	C_1							128.97	129.17	129.45
	C_3, C_5							115.94	116.45	116.53
	C=O							173.25	173.13*	
Cys	αCH								50.27	45.69*
	βCH_2								37.04	41.41
	C=O								171.74	
Z	C_1	138.59	138.51	138.51	138.51	138.51	138.58	138.59		
	C_2, C_6	129.77	129.85	129.52	129.85	129.97	129.97	129.97		
	C_3, C_5	128.97	129.05	128.38	129.17	129.25	129.25	129.17		
	C_4	128.48	128.46	128.18	128.38	128.38	128.38	128.38		
	CH_2	68.37	67.45	67.25	67.17	67.05	66.85	67.17		
S-Bzl	CH_2			38.38	34.18	33.98	34.06	34.06	34.06	
	Arom			139.98	139.98	140.10	140.10	140.10		
	Arom			130.44	130.56	130.56	130.56	130.56		
	Arom			129.96						

In addition, the CH_2 and CH_3 of O-Et of I resonates at 61.88 and 15.58 ppm, respectively; the CH_2 of O-Bzl of VII at 70.83 and the CH_2 of S-Bzl of VIII at 35.17 ppm.

* Assignment uncertain.

H-Cys(Bzl)-Pro-Leu-Gly-NH_2, IX; HBr·H-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, X; HBr·H-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, XI; HBr·H-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, XII; HBr·H-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2,XIII. CMR spectra were recorded at 37°C on a Varian XL-100-15 spectrometer operating at 25.16 MHz in the pulsed Fourier transform mode with complete proton decoupling.

RESULTS AND DISCUSSION

Assignment of the C-13 resonances from individual carbons in the tripeptides I and II was made by comparison with the resonance positions for amino acids in neutral D_2O (5,6) and protected amino acids in DMSO-d_6(6,7). The major changes in chemical shift between I and II are in the glycine residue. The α carbon shifts downfield 1.0 ppm while the carbonyl resonance moves downfield 1.2 ppm; this is expected since amino groups exert little effect on the carbonyl resonance whereas formation of a methyl ester causes an upfield shift (4).

In I and II two lines appear for each of the α, β, and δ carbons of Pro (Fig. 1). The γ carbon resonance is close to one of the δ carbon resonances of Ile and a second line may be obscured by this resonance. This doubling suggests cis-trans isomerism about the Z-Pro bond, as also seen by 220 MHz proton magnetic resonance (8). It was found that cis and trans isomers about the Z-Pro bond were equally favored. A 100 MHz proton spectrum of peptide II was similar to that previously reported (1,8).

In peptide III only one resonance is visible for each carbon of Pro. The 220 MHz proton spectrum does not demonstrate the presence of cis and trans isomers of Pro. It has been postulated (8) that an interaction occurs between the lone pairs on the sulfur of Cys and the carboxamide-NH_2 in III. Since no changes other than in the Pro residues are seen on going from peptide II to peptide III, the C-13 spectra provide no evidence for such an interaction.

In peptide IV, the C-13 resonance of the Cys carbonyl group moves up-

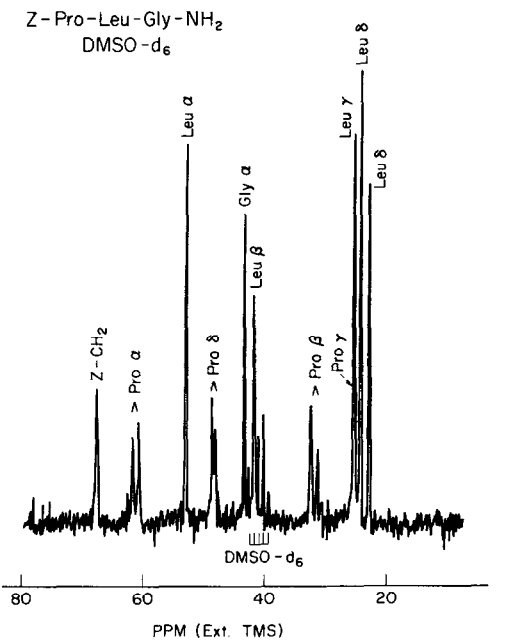

Fig. 1

CMR spectrum of Z-Pro-Leu-Gly-NH_2 in DMSO-d_6. Transients (14,000) were accumulated with an acquisition time of 0.4 sec and a pulse width of 40°.

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field 0.5 ppm while the α carbon moves upfield 1.8 ppm. Only one resonance is seen for the carbonyl carbons of Asn; it is broader and more intense than the other carbonyl resonances. The phenomenon also occurs in the protected amino acid (6).

In peptide V, the Asn residue undergoes the largest changes relative to IV. The α and β carbons move upfield by 2.0 and 0.3 ppm respectively, and the carbonyl carbon moves downfield 0.2 ppm. The Gln residue in V, unlike the Asn, shows two peaks in the carbonyl region. This is not unexpected; Asn (free amino acid in D_2O) carbonyl resonances are separated by 1.2 ppm while the carbonyl resonances in Gln (free amino acid in D_2O) are separated by 3.6 ppm.

In successive additions of N-terminal residues (peptides VI-VIII)the main changes are observed in the penultimate residue. In general the α

carbon moves downfield 2.0 to 2.4 ppm, and the β and carbonyl carbons show upfield or downfield shifts of less than 0.5 ppm.

The deprotected peptides (X-XII) were examined in DMSO-d_6 and D_2O. By comparing spectra of the protected (III-VII) and deprotected peptides (IX-XIII) in DMSO-d_6 it was possible to evaluate the effect of the protecting group on the carbon chemical shifts of the peptides. Comparison of the data for X-XII obtained in the two solvents allows an estimate of the non-conformational solvent effects. These effects can then be taken into account when considering the possibility of conformational differences for oxytocin in the two solvents.

The spectra of protected and deprotected peptides in DMSO-d_6 are identical except for the resonances of the N-terminal residues which in the protected peptides are shifted downfield by the Z-group. For example, in the protected hexapeptide (V) the α carbon of the terminal Gln is 2.8 ppm to lower field, the β carbon 1.2 ppm and the γ carbon is 1.0 ppm to lower field than in the deprotected peptide.

In the acyclic peptides the order of chemical shifts is the same as that of the constituent amino acids. No crossover of resonances occurs.

Cyclization of the fully deprotected nonapeptide to yield oxytocin gives rise to numerous spectral changes (Fig. 2). The major changes occur in the α and the carbonyl carbons. In general, cyclization causes a downfield shift of resonances originating from carbon atoms of the cyclic moiety; slight changes are also detected in resonances of β carbons. Those of Cys appear to move downfield as the ring is closed. It is not possible at present to make unequivocal assignment of all the carbonyl resonances in the hormone. These assignments await the study of a series of cyclic neurohypophyseal hormone analogs.

The spectral changes for oxytocin in DMSO-d_6 and D_2O seem to parallel those induced by solvent on the constituent oligopeptides. Thus, the CMR spectra provide no evidence for significant conformational differences in

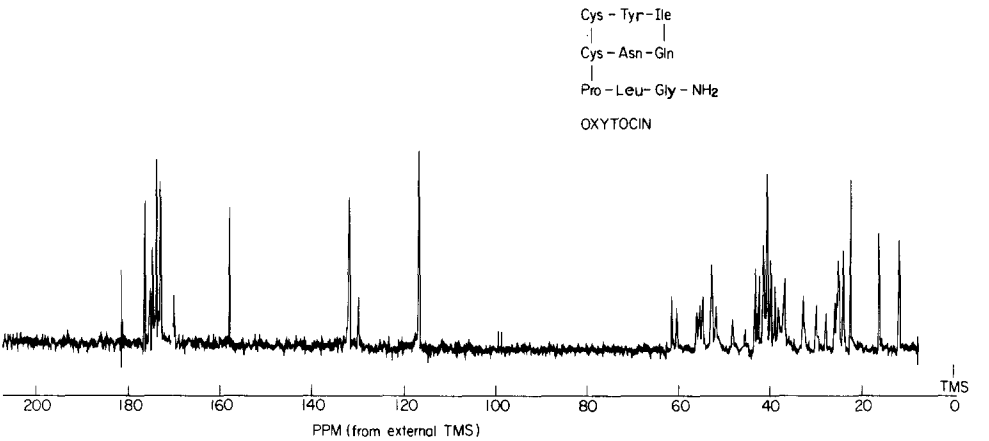

Fig. 2

CMR spectrum of oxytocin in DMSO-d_6. Transients (84,000) accumulated with an acquisition time of 0.4 sec, a cycle time of 1.0 sec and a pulse width of 90°.

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the two solvents. However, it would be premature to assert that there are no conformational differences in the two solvents since the conformational sensitivity of the C-13 resonances of amino acids has not yet been well established.

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