Volume 250, number 2, 405–410 FEB 07317 July 1989

Conformational behavior of fragments of adrenocorticotropin and their antisense peptides determined by NMR spectroscopy and CD spectropolarimetry

Elias S. Najem*, Angela Corigliano-Murphy° and James A. Ferretti

*Howard Hughes Medical Institute, °Laboratory of Cell Biology and Laboratory of Chemistry, NHLBI, National Institutes of Health, Bethesda, MD 20892, USA

Received 2 March 1989; revised version received 4 May 1989

An 'antisense' peptide ('HTCA'), whose sequence was generated by reading the antisense RNA sequence corresponding to ACTH(1–24) was shown to bind ACTH(1–24) with a K_d of 0.3 nM in a solid-matrix binding assay [(1986) Biochem. J. 234, 679–683]. Two-dimensional NMR spectra were used to examine the conformational behavior in methanol and in water solution of two fragments of adrenocorticotropin, ACTH(1–24) and ACTH(1–13), as well as their antisense peptides, HTCA and HTCA(12–24). The conformations are extended chains in these solutions, both as isolated molecules and when mixed with their antisense complements. The K_d values are greater than 1 mM.

Peptide, antisense; Adrenocorticotropic hormone; NMR, 2D; CD

1. INTRODUCTION

The mechanism of action of peptide hormones depends, in part, on specific interaction with cell surface-associated receptors. Such physical techniques as NMR spectroscopy have been applied to the determination of the structures of peptides and their receptors in solution. Such studies are often limited because of the quantities required and the difficulties involved in obtaining purified receptor. Thus, there is a need for model systems that may provide clues to the important determinants for recognition between peptides and their receptors.

Recently, Bost et al. [1] proposed a 'new theory' for the interactions between peptides and proteins. It is based on the observation that there is a general tendency for codons for hydrophobic amino acid residues to have complements (i.e. antisense sequences) which code for hydrophilic amino acid residues, and vice versa. Citing evidence that 'amphiphilic conformations' may be involved in peptide-receptor interactions [2], they proposed that an implication of this pattern in the genetic code is that the RNA sequence complementary to the mRNA sequence coding for a particular peptide, i.e. antisense RNA, read in either the 5′ to -3′ or 3′ to -5′ direction, would code for an antisense peptide capable of binding that peptide. This idea was first tested on the 1–24 fragment of the 39 amino acid residue peptide, adrenocorticotropic hormone (ACTH). Two antisense peptides to ACTH(1–24) were found to bind ACTH(1–24) with dissociation constants, K_d, as low as 0.3 nM, in a solid-matrix binding assay [1,3]. Furthermore, antibodies to one of these peptides, denoted HTCA, derived from reading the antisense RNA sequence in the 5′-to-3′ direction, were shown to interact with a putative ACTH receptor on mouse adrenal tumor (Y-1) cells, thus implying a structural similarity between this antisense peptide and a receptor for ACTH [4].

In an attempt to determine the nature of these interactions, we obtained the NMR and CD spectra of both the 1–24 and 1–13 fragments of ACTH and

Correspondence address: E.S. Najem, Howard Hughes Medical Institute, National Institutes of Health, Bethesda, MD 20892, USA

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

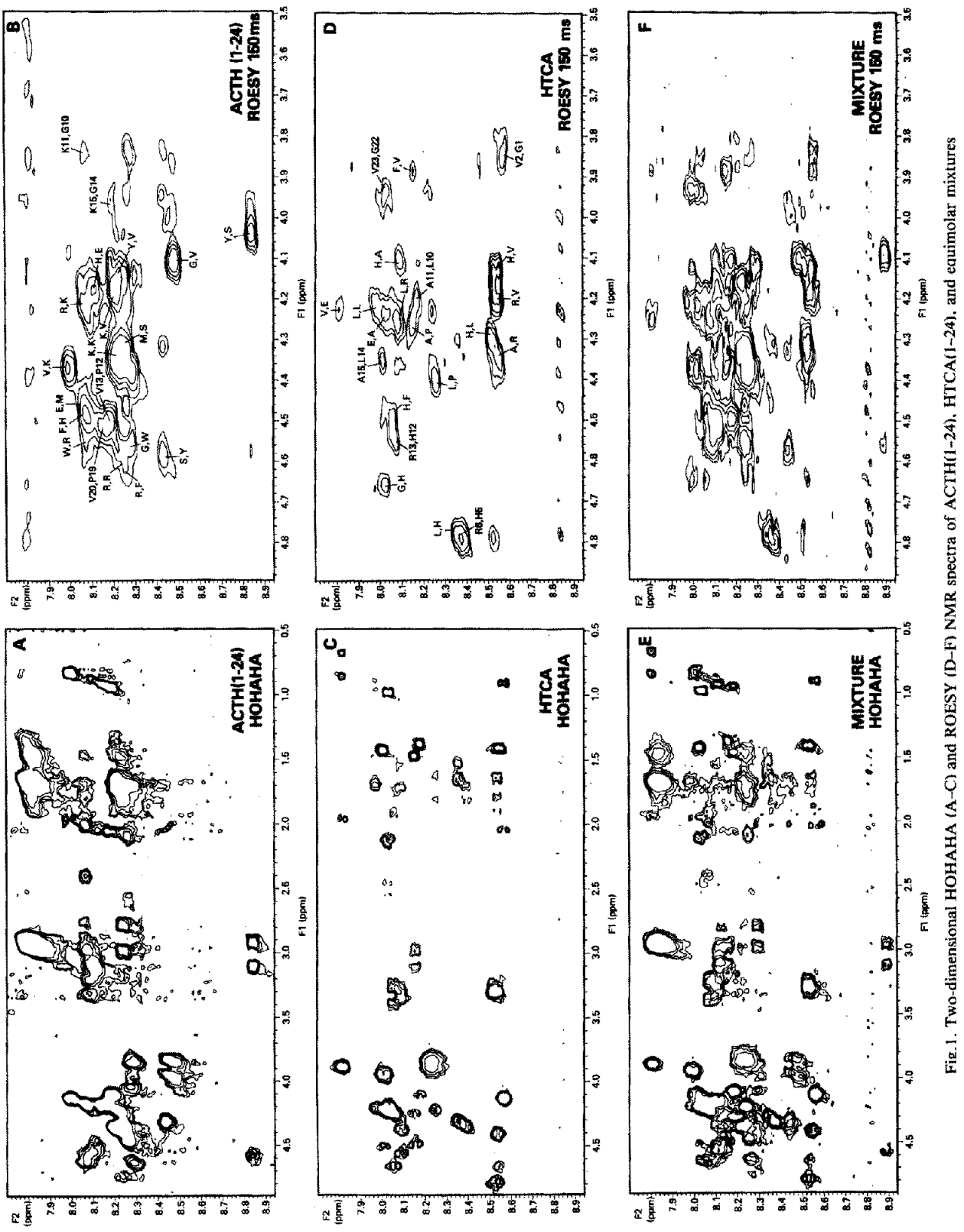

Fig. 1. Two-dimensional HOHAHA (A–C) and ROESY (D–F) NMR spectra of ACTH(1-24), HTCA(1-24), and equimolar mixtures

their respective antisense peptides in methanol and in aqueous solution. The 24-residue fragments were also studied in the presence of SDS micelles. The solution conformations of ACTH(1–13) and (1–24), and their corresponding antisense peptides, do not change when the sense and antisense peptides are mixed together. Our solution studies show no evidence of binding of ACTH(1–24) and ACTH(1–13) to their respective antisense peptides.

2. MATERIALS AND METHODS

2.1. *Synthesis of antisense peptides*

The sequence of HTCA is H_2N-GVHLHRAPLLAHRLAP AEVFHGVR-COOH, which corresponds to reading the antisense RNA sequence complementary to the mRNA sequence for ACTH(1–24) in the 5′-3′ direction. The antisense peptide to ACTH(1–13) is residues 12–24 of HTCA. Both of these peptides were synthesized on an Applied Biosystems model 430A peptide synthesizer using *t*boc chemistry. The crude peptide was loaded onto a Sephadex G-25 column (2 × 90 cm) and eluted with a 5% acetic acid solution. Amino acid analysis and ^{252}Cf mass spectrometry confirmed the identity of the peptides.

2.2. *NMR spectroscopy*

The individual ACTH(1–13) (Peninsula Labs) and HTCA(12–24) peptides were made up to be 4 mM in 10% D_2O/90% H_2O. The solution of the mixture of the two peptides contained 5 mM of each individual peptide in 10% D_2O/H_2O. The solution pH of ACTH(1–13) was pH 2.9 (± 0.05), pH 4.3 (± 0.05) for HTCA(12–24) and pH 4.0 (± 0.05), for the mixture.

Both 24-residue peptides, ACTH(1–24) (Sigma) and HTCA, and their mixture were prepared as 4 mM solutions in d_3-methanol with the addition of the minimum amount of conc. DCl required for complete dissolution. In the case of the mixture, the concentration of each inidividual peptide was 4 mM.

The ^1H NMR spectra of the various peptides were obtained at 18°C on a Varian XL-300 spectrometer equipped with a 5-mm dual probe. Resonance assignments, as well as conformational and binding studies, were carried out using a combination of 2D

Table 1

^1H-NMR chemical shifts for ACTH(1–24) in d_3-methanol at 18°C

Residue	δ (ppm)					
	NH	α	β	Γ	δ	ε
S1	—	4.04	3.93			
Y2	8.83	4.59	3.09,2.92			
S3	8.43	4.30	3.95,3.82			
M4	8.27	4.46	2.14,2.63			
E5	8.07	4.19	2.04,1.96	2.43		
H6	8.12	4.48	3.12,3.07			
F7	8.06	4.61	2.85,2.75			
R8	8.27	4.60	1.80	1.70	3.20	
W9	8.09	4.55	3.31,3.20			
G10	8.28	3.85,4.04				
K11	8.06	4.62	1.76,1.69	1.47,1.40	1.80	3.20
	7.46	(ε-amino)				
P12	—	4.50	2.15	1.95	3.66,3.78	
V13	8.18	4.10	2.05	0.95		
G14	8.47	3.95,3.82				
K15	8.19	4.35	1.70,1.64	1.45,1.35	1.70	2.90
	7.82	(ε-amino)				
K16	8.22	4.22	1.72	1.49	1.72	3.12
	7.40	(ε-amino)				
R17	8.06	4.62	1.76,1.69	1.69	3.20	
	7.46	(δ NH)				
R18	8.27	4.60	1.86	1.70	3.20	
	7.46	(δ NH)				
P19	—	4.50	2.15	1.95	3.66,3.78	
V20	8.13	4.18	2.07	0.93		
K21	8.21	4.36	1.68	1.42	1.68	2.90
V22	7.99	4.14	1.95	0.84		
Y23	8.24	4.75	2.98,2.79			
P24	—	4.35	2.22	1.96	3.72,3.40	

HOHAHA [5] and ROESY [6] spectroscopy and 1D difference experiments.

2.3. *CD spectropolarimetry*

CD measurements were performed on a Jasco model J-600 spectropolarimeter using sample chambers with a 0.1 mm path length. The 13-residue sense and antisense ACTH peptides were prepared as 0.1 mM solutions in both H_2O and methanol. The mixture of the two contained an equimolar amount of each peptide, with the total peptide concentration being 0.1 mM. In the case of the 24-residue peptides, CD spectra were obtained for solutions containing 0.0125, 0.025 and 0.05 mM of each peptide in water at pH 5, methanol, and phosphate-buffered saline (pH 7.2). Again, in each case, the mixtures of the two peptides contained equimolar amounts of the individual peptides. Results are reported as mean residue ellipticity ($\theta \cdot dM^{-1}$) vs wavelength in each case.

3. RESULTS

The spectra are shown in fig.1 and complete resonance assignments of both ACTH(1–24) and HTCA in d_3-methanol are presented in table 1. In the ROESY spectra of the individual peptides (fig.1B,D), using a 150 ms mixing time, no medium- or long-range interactions were observed. This suggests that both ACTH(1–24) and HTCA are present primarily as random coils or in extended chain conformations in methanol. The CD spectra of these peptides in methanol are also consistent with these observations (fig.2A), although HTCA shows additional negative ellipticity around 222 nm. Similar CD spectra were obtained ellipticity around 222 nm. Similar CD spectra were obtained in phosphate-buffered saline at pH 7.2 (fig.2B). Fig.1E and F shows the HOHAHA and ROESY spectra of an equimolar mixture of ACTH(1–24) and HTCA in methanol. No changes in chemical shift of any of the resonances of either peptide in the mixture are observed, as compared to the spectra of the isolated peptides. These peptides assume the same conformation in the mixture as they do as isolated molecules, which suggests the lack of any interaction between the two peptides.

The CD spectra of the mixture of the sense and antisense peptides are consistent with the NMR data presented above. Fig.2A shows the CD spectrum of the mixture of the two peptides in methanol superimposed with the spectra of the individual peptides and the sum of the spectra of the individual peptides. (The concentration of the individual peptides in the mixtures was half that of the isolated peptides, so that the total concentration of peptide was the same in each case. The effects of any concentration-dependent photomultiplier tube voltage artifacts are eliminated.) The spectrum of the mixture is the same as the sum of the individual spectra. At the concentrations used, this implies a dissociation constant greater than 10^{-3} M, i.e. less than 5% of the peptides would be in the bound state. The CD spectra of the two peptides obtained in phosphate-buffered saline at pH 7.2 also show no indication of binding (fig.2B). Similar results were obtained in water at pH 5 and 0.16 M KCl at pH values of 3, 5 and 7. Studies were also carried out in the presence of 0.5% SDS [7] in phosphate-buffered saline at pH 7.2 (fig.2C). Con-

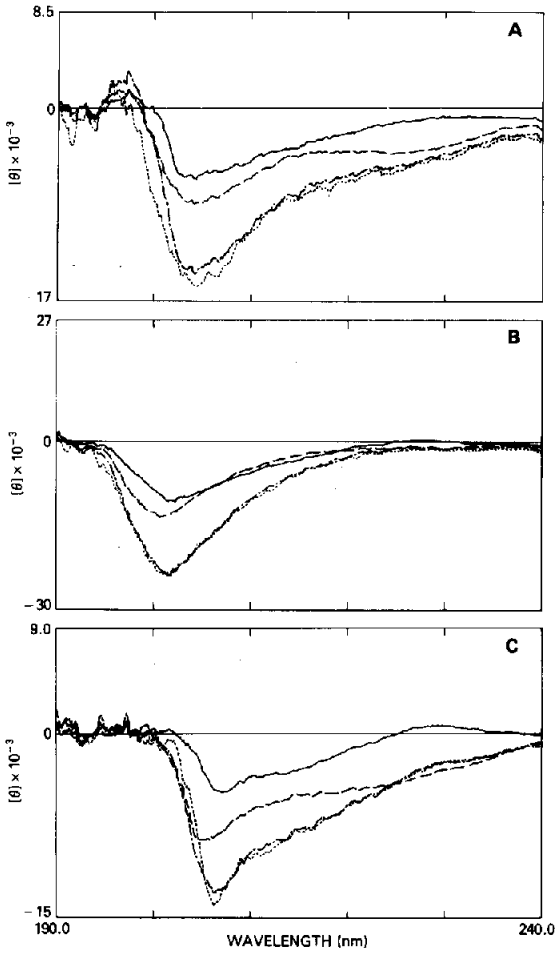

Fig.2. Series of CD spectra of ACTH(1–24), HTCA(1–24), equimolar mixtures of the two peptides, and the sum of the ellipticities of the individual peptides in (A) methanol, (B) phosphate-buffered saline (PBS) and (C) 0.4% SDS in PBS.

Table 2

^{1}H-NMR chemical shifts for HTCA(1–24) in d_3-methanol at 18°C

Residue	δ (ppm)					
	NH	α	β	Γ	δ	ε
G1	8.23	3.86				
V2	8.57	4.12	2.04	0.91		
H3	8.54	4.74	3.29,3.26			
L4	8.36	4.29	1.64	1.64		
H5	8.52	4.77	3.29,3.23			
R6	8.39	4.35	1.82	1.70	3.23	
	7.56 (δ NH)					
A7	8.54	4.67	1.41			
P8		4.39	2.32	2.00	3.76	
L9	8.25	4.20	1.81	1.64	0.95	
L10	7.98	4.20	1.7	1.65	0.95,0.90	
A11	8.18	4.09	1.37			
H12	8.09	4.55	3.36,3.29			
R13	8.06	4.25	1.91	1.70	3.22	
	7.56 (δ NH)					
L14	8.08	4.37	1.73	1.54		
A15	8.01	4.49	1.43			
P16		4.29	2.32	1.98	3.75	
A17	8.15	4.24	1.47			
E18	8.03	4.23	2.15	2.55,2.47		
V19	7.82	3.88	1.96	0.86,0.67		
F20	8.18	4.48	3.11,2.98			
H21	8.06	4.65	3.48,3.26			
G22	8.01	3.94				
V23	8.03	4.21	2.11	0.98		
R24	8.54	4.40	1.90,1.79	1.64	3.19	
	7.46 (δ NH)					

siderably more order in the structure of HTCA was observed under these conditions, as seen by the large value of the ellipticity around 215–225 nm. Again, no difference is observed between the spectrum of the mixture of the sense and antisense peptides and the sum of the spectra of the individual peptides.

In order to determine whether any binding between the two peptides could be detected when one of the peptides was in large excess, one-dimensional NMR spectra were obtained of mixtures of the two peptides containing either a 15- or 5-fold molar excess of HTCA over ACTH. In each case the concentration of HTCA was 7.5 mM. The concentration of ACTH was either 0.5 or 1.5 mM. Spectra were obtained in both d_3-methanol and D_2O. The spectra of the mixtures and the sum of the spectra of equivalent amounts of the individual peptides (not shown) were identical.

4. DISCUSSION

Although ACTH(1–24) and HTCA are reported to bind in a solid-matrix binding assay [1,3], we find no evidence for binding under various solvent conditions. Presumably, any mutual affinity of the two peptides is insufficient to overcome solvation energies. Since the initial report by Bost et al. [1], other groups have reported studies involving sense-antisense interactions with mixed results [8–12]. A lack of any significant binding of ACTH(1–24) and ACTH(1–13) with their respective antisense peptides in solution would preclude the use of these peptides as model systems for studying receptor recognition. The origin of the difference between the solution and solid-phase dissociation constants is hard to understand. Additional studies to clarify this discrepancy are needed. Our results, together with the difficulty in understanding the origin of an

attractive force between a hydrophobic and hydrophilic residue, question the significance of the hydrophobic-hydrophilic complementarity model of peptide recognition.

Acknowledgements: The authors wish to thank Drs Marshall Nirenberg, Irwin Chaiken, Peter Roller, and Henry Fales for helpful discussions and reading the manuscript. E.S.N. is a Howard Hughes Medical Institute Research Scholar.

REFERENCES

[1] Bost, K.L., Smith, E.M. and Blalock, J.E. (1985) Proc. Natl. Acad. Sci. USA 82, 1372-1375.
[2] Kaiser, E.T. and Kezdy, F.J. (1984) Science 223, 249-255.
[3] Blalock, J.E. and Bost, K.L. (1986) Biochem. J. 234, 679-683.
[4] Bost, K.L. and Blalock, J.E. (1986) Mol. Cell. Endocrinol. 44, 1-9.
[5] Bax, A. and Davis, D.G. (1985) J. Magn. Reson. 65, 355-360.
[6] Bothner-By, A.A., Stephens, R.L., Lee, J., Warren, C.D. and Jeanloz, R.W. (1984) J. Am. Chem. Soc. 106, 811-813.
[7] Mayo, K.H., De Marco, A., Menegatti, E. and Kaptein, R. (1987) J. Biol. Chem. 262, 14899-14904.
[8] Rasmussen, U.B. and Hesch, R.D. (1987) Biochem. Biophys. Res. Commun. 149, 930-938.
[9] Görcs, T.J., Gottschall, P.E., Coy, D.H. and Arimura, A. (1986) Peptides 7, 1137-1145.
[10] Johnson, H.M. and Torres, B.A. (1988) J. Immunol. 141, 2420-2423.
[11] Knigge, K.M., Piekut, D.T. and Berlove, D. (1988) Neurosci. Lett. 86, 269-271.
[12] Shai, Y., Flashner, M. and Chaiken, I.M. (1987) Biochemistry 26, 669-675.