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A 500 MHz study of peptide T in a DMSO solution

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Peptide T, an octapeptide of sequence ASTTTNYT that binds to human T cells, was studied as a zwitterion in $DMSO_{d6}$ solution by means of proton NMR spectroscopy at 500 MHz. The unusual dispersion of the resonances of residues of the same type (T) makes it possible to assign all resonances to specific residues by means of several 2D techniques. The non-random nature of the conformation is substantiated by the observation of sequential nuclear Overhauser enhancements (NOEs). The low value of the temperature coefficient of the chemical shift of the NH of T^8 and a diagnostic NOE between the NHs of T^7 and T^8 hint that a β-turn including T^5, N^6, Y^7 and T^8 is a prominent conformational feature in solution. The ring current high field shifts of the methyl group and of the NH of T^8 are consistent with an interaction with the side-chain of Y^7, favoured by the β-turn.

HIV; Peptide conformation; NMR

1. INTRODUCTION

It has been recently shown [1] that a simple octapeptide, deduced from a computer analysis of the envelope protein HIV gp120 and termed 'peptide T', may play an important role in HIV attachment. The possible clinical use [2] of peptide T in the control and treatment of AIDS poses a cogent problem of drug design, with the hope of finding more potent and/or more resistant analogs [3]. SAR problems in the field of biologically active peptides are best tackled with a preliminary conformational analysis of the parent peptide. Medium-sized linear peptides are usually very difficult to study since they tend to exist as a complex mixture of conformers of similar energy. Besides, the combination of external (tumbling) and internal motions leads to correlation times that minimize all NOEs [4]. In the case of peptide T we feared that the unusually uniform composition, i.e. four T residues (and six OH bearing residues) out of eight, might render rather difficult even NMR assignment. On the contrary, we found that the NOESY spectrum of the peptide T contains intraresidue and sequential NOEs, consistent with a sizeable population of fairly rigid conformer(s). These effects also allowed a unique identification of all residues.

Both chemical shift and variable temperature data point to the relevant role (in the mixture of conformers) of a type 1 β-turn involving the four C-terminal residues.

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Abbreviations: HIV, human immunodeficiency virus; gp120, HIV envelope glycoprotein; $DMSO_{d6}$, deuterated dimethylsulfoxide; AIDS, acquired immunodeficiency syndrome; NOE, nuclear Overhauser enhancement; NMR, nuclear magnetic resonance; A, S, T, N, Y, one-letter code for alanine, serine, threonine, asparagine and tyrosine, respectively; TMS, tetramethylsilane

2. MATERIALS AND METHODS

Peptide T, H-Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr-OH, was synthesized by classical solution methods and purified by means of silica gel column chromatography, eluted with *n*-butanol/acetic acid/water (6:2:2), and reverse-phase HPLC on C_{18}, eluted with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid. The final product, m.p. 175–177°C, $[\alpha]_D^{25}$ (c = 1, DMF) = −2.2, crystallized by ethyl acetate. It was isolated as trifluoroacetate.

$DMSO_{d6}$ (99.8% atom ^2H) was purchased from C. Erba (Milan, Italy).

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NMR spectra were obtained at 500 MHz in the Fourier mode using a Bruker WM-500 spectrometer. Double-quantum-filtered (DQF) COSY [5] and NOESY [6] spectra were run in phase-sensitive mode. Quadrature detection in ω_1 was achieved by time proportional phase incrementation of the initial pulse [7]. Data block sizes were 2048 addresses in t_2 and 512 equidistant t_1 values, adding 80 transients for each t_1. The relaxation period was 1.5 s between scans, and the spectral width was 6000 Hz, which yields a resolution of 5.6 Hz/point for both experiments. Double-quantum (DQ) experiments were run according to Mareci and Freeman [8], selecting a 135° detection pulse and an evolution period of 1/8 J. 96 transients were collected for each t_1, using a sweep of 6000 Hz in ω_2. The time domain matrix was 512 × 2048. Before Fourier transformation, the time domain matrices were all multiplied by a 6°-shifted sine-bell function in both domains.

Chemical shifts are reported as δ values, in ppm from internal TMS.

Table 1

Chemical shifts (ppm, referred to internal TMS) and temperature coefficients of NH resonances of peptide T 2 mM in $DMSO_{d6}$ at 298 K

Residue	NH	$C_\alpha H$	$C_\beta H$		$\Delta\delta/\Delta T \times 10^3$
Ala[1]	–	3.81	1.32		
Ser[2]	8.69	4.42	3.66	OH 5.16	−5.4
Thr[3]	7.90	4.31	4.09	CH_3 1.03	−4.0
Thr[4]	7.85	4.30	4.07	CH_3 1.03	−2.8
Thr[5]	7.69	4.25	4.03	CH_3 1.00	−2.3
Asn[6]	8.02	4.51	2.36	NH_2 6.97	
			2.56	7.40	−3.3
Tyr[7]	8.00	4.37	2.74	Ar 6.64	
			2.95	7.03	−3.3
Thr[8]	7.54	3.96	3.96	CH_3 0.94	−0.65

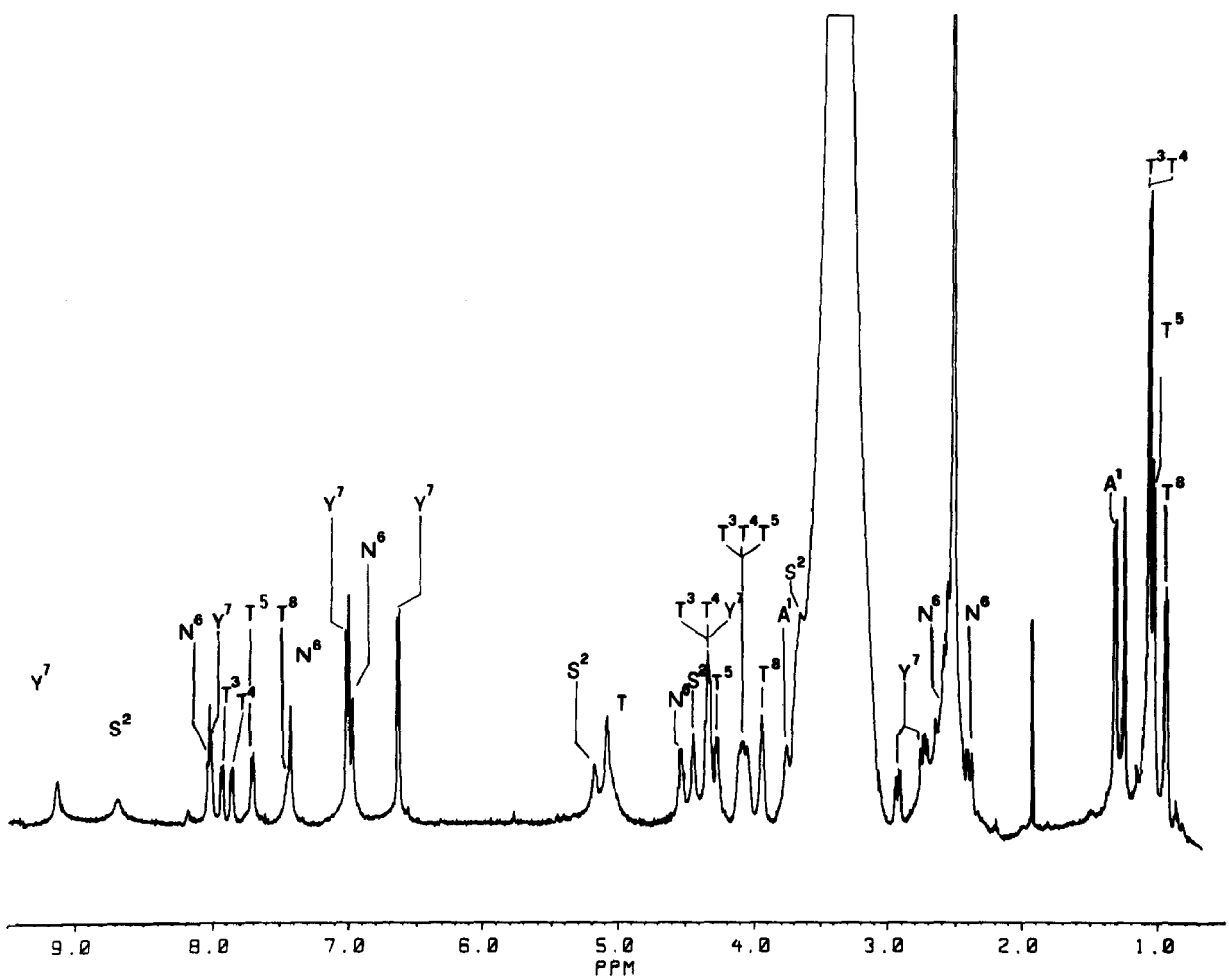

Fig.1. 500 MHz proton NMR spectrum of 2 mM peptide T in $DMSO_{d6}$ at 298 K. One letter code for amino acids has been used throughout to label resonances belonging to the different residues. The ppm scale is referred to internal TMS.

3. RESULTS AND DISCUSSION

Fig.1 shows the 500 MHz 1D spectrum of peptide T in $DMSO_{d6}$ at 298 K. It is interesting to note that, in spite of the presence of four T residues (out of a total of eight residues), the resonances of the NH groups are all separated and spread over a considerable chemical shift range (table 1). Two of these resonances (S^2 and T^8) are definitely broader than the other five, indicating that these residues might occupy a singular conformational position. In the aliphatic region, the side chains of the four threonines give rise to three distinct CH_3 resonances, indicating that only two of these residues, whose methyls resonate at 1.03 ppm, are in a similar environment. One of the other two

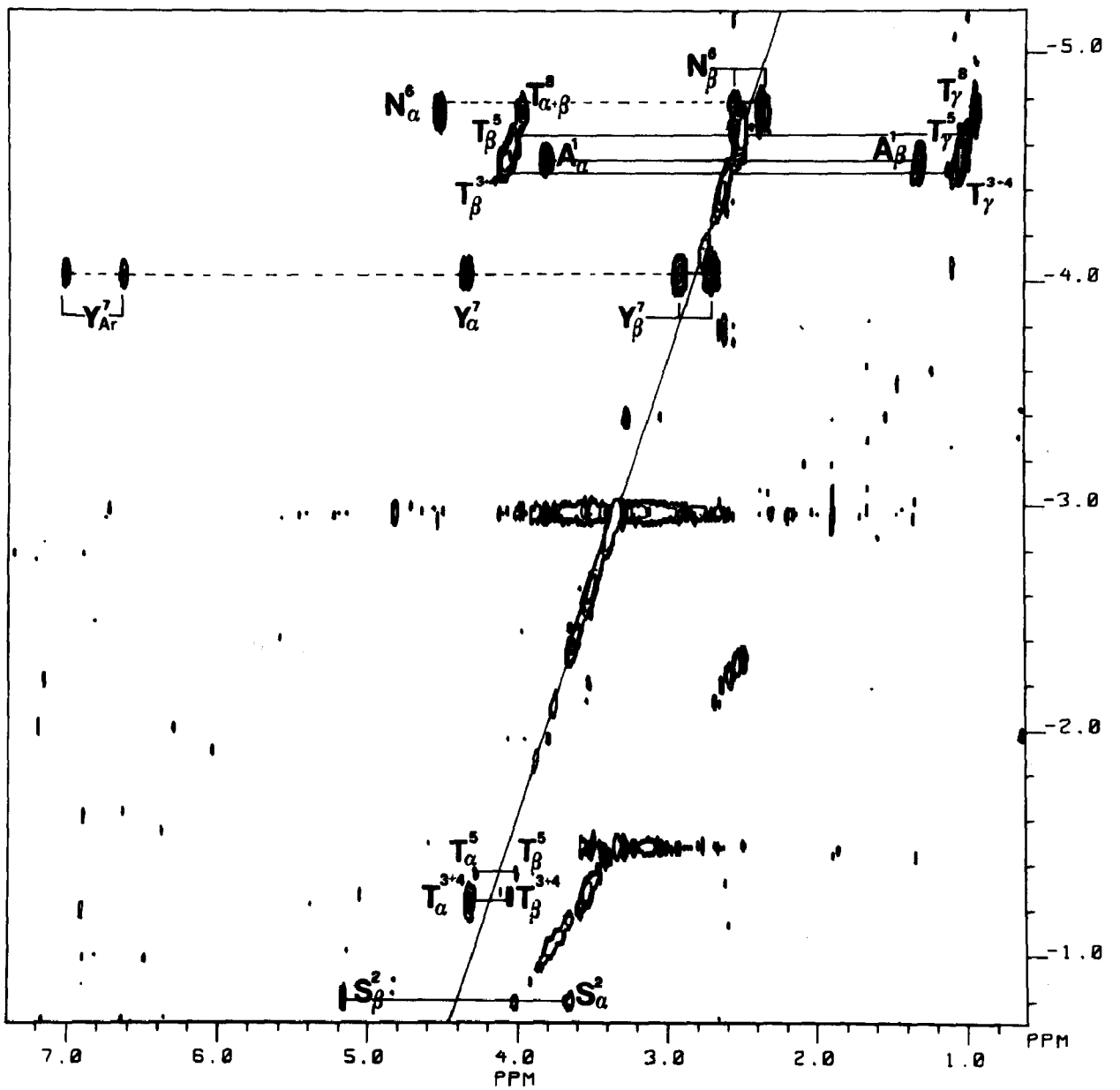

Fig.2. 500 MHz DQ-spectrum of 2 mM peptide T in $DMSO_{d6}$ at 298 K. Data are presented as absolute value contour plot. The solid line indicates the DQ diagonal ($\omega_1 = 2\,\omega_2$). The shown spectral region contains direct (——) and long range (– – –) connectivities for non-labile protons. The chemical shifts scale is referred to internal TMS.

CH_3 doublets, at 0.94 ppm, is significantly shifted to high field compared to T methyl in model peptides, a phenomenon reminiscent of ring-current high field shifts in proteins.

All these simple chemical shift data point to a non-random conformational state. However, a more detailed analysis requires a complete resonance assignment.

Owing to the severe problems of resonance superpositions in the α-CH region, due to the numerous α- and β-CH peaks of T and S residues, the assignment work was carried out by using a combination of DQF COSY and DQ experiments.

Fig.2 shows the non-exchangeable proton portion of the DQ contour plot, in which the connectivities for all the side-chain resonances could be identified, and are all labeled. Long-range couplings between the aromatic and the α- and β-protons of Y^7 are also observed (Y^7 diagram).

Conformational studies require sequential assignments, which are usually obtained through the detection of NOEs between pairs of α-CH and NH protons belonging to adjacent residues. Most medium-sized linear peptides, at high field, give very small NOEs, owing to the unfavorable value of $\omega\tau_c$ [4] and to the lack of well defined structural features.

Surprisingly, the NOESY spectrum of fig.3 shows effects between the amide and the α-protons (and β-protons in the case of Ts) of adjacent residues. Furthermore, effects between NHs of T^4 and T^5, and Y^7 and T^8 are also observed.

These findings allow not only a complete assignment, but indicate also the presence of well defined and fairly rigid conformers. The inclusion of residues 4–8 in a single, ordered structure could amount to proposing a 4–8 helical segment (either α or 3_{10}). In fact it is not possible to rule out an effect between the superposed NHs of N^6 and Y^7. However, the absence of a T^5 NH-N^6 NH cross-peak makes the simpler hypothesis of a 5–8 β-turn more tenable [9].

Table 1 summarizes the assignments of all backbone protons, together with the temperature coefficients of the labile protons. The very low value of the T^8 NH chemical shift, in the range 298–330 K, is suggestive of the involvement of this proton in an intramolecular hydrogen bonding [10].

The most likely cyclic structure is a type 1 β-turn

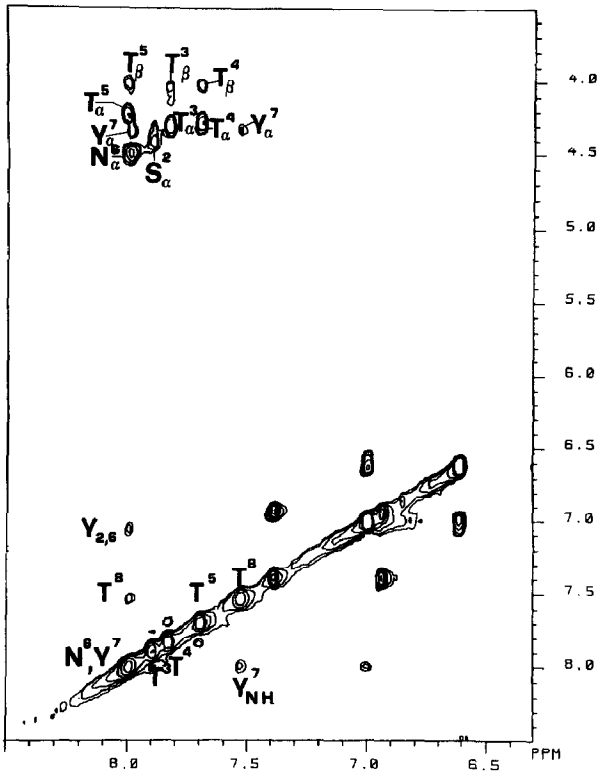

Fig.3. Low-field region of a 2D NOESY spectrum of 2 mM peptide T in $DMSO_{d6}$ showing cross-peaks from amidic and aromatic protons to NH, $C_\alpha H$ and $C_\beta H$. Mixing time = 500 ms, T = 298 K.

in which T^8 is bonded to T^5 CO. This C_{10} structure is consistent with the high-field shifts of T^8 CH_3 and NH chemical shifts, since it favors the interaction of these groups with the aromatic ring of Y^7.

4. CONCLUSION

Peptide T shows an unusual degree of conformational order in $DMSO_{d6}$ solution. Diagnostically relevant chemical shift, NOE and variable temperature data are consistent with a β-turn including T^5, N^6, Y^7 and T^8.

This conformation is probably not the only one present in solution, but it appears the only one observable owing to the non-linear dependence of NOE on interatomic distances. Residues 5–8 had already been found to play a crucial role in potent pentapeptide analogs [3]. New linear and cyclic analogs designed to enhance the stability of the proposed β-turn are under investigation in our laboratory.

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