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SIMULTANEOUS OBSERVATION OF POSITIVE AND NEGATIVE NUCLEAR OVERHAUSER EFFECTS IN OLIGOPEPTIDES DUE TO SEGMENTAL MOTION

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SUMMARY: Nuclear Overhauser effect (NOE) studies of the symmetrical cystine peptides Boc-Cys-$(Val)_n$-Trp-OMe (n=1-3) in dimethylsulfoxide, Boc-Cys-$(Val)_n$-Trp-OMe have resulted in the simultaneous observation of both positive and negative NOEs. Positive NOEs are observed on the Trp C^{β}2H and C4H protons of the indole ring upon irradiation of Trp C^{α}H and $C^{\beta}H_2$ resonances in the peptides where n=1 and 2. Negative NOEs are observed between backbone NH and C^{α}H protons. The magnitudes of the observed NOEs are sensitive to changes in molecular size and solvent viscosity. The results demonstrate that NOEs may be a useful probe of sidechain segmental motion in oligopeptides.

The sign of the nuclear Overhauser effects (NOEs) observed between spatially proximate protons is dependent on the product of the Larmor precesion frequency (ω) and the correlation time (τ_c), modulating the magnetic dipole-dipole interaction (1,2). For solutions of low molecular weight systems in non-viscous solvents, at normally accessible spectrometer frequencies, $\omega\tau_c < 1$ and the observed NOEs are invariably positive (2). However, in the case of macromolecules which tumble relatively slowly in solution $\omega\tau_c$ is often > 1 and negative NOEs are obtained (3,4). In principle, in complex systems local motions may dominate dipole-dipole relaxation processes such that the observed NOEs are no longer dependent on the overall tumbling of the molecule, but are determined instead by segmental mobility. We describe in this report the observation of both positive and negative interproton NOEs in symmetrical cystine peptides, which provide a direct diagnostic for segmental motion of amino acid sidechains in these systems.

MATERIALS AND METHODS

The peptides **1-3** were synthesized by conventional solution phase procedures and purified by silica gel column chromatography. Peptide

Abbreviations used: Boc, t-butyloxycarbonyl; OMe, methyl ester; NOE, nuclear Overhauser effect.

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homogeneity was established by HPLC on a Lichrosorb RP-18 column using methanol-water gradients and all samples were fully characterized by 270 MHz ^{1}H NMR. Difference NOE experiments were carried out on a Bruker WH-270 NMR spectrometer equipped with an Aspect 2000 computer, as described earlier (5). Reported spectra were obtained using 500 scans for on and off resonance spectra. Peptide concentrations ranged from 10 to 40 mg/ml in $(CD_3)_2SO$. All spectral assignments were made using two-dimensional correlated spectroscopy and sequential NOEs (4).

RESULTS AND DISCUSSION

Figures 1 and 2 show the difference NOE spectra obtained by irradiation of the Trp $C^{\alpha}H$ (4.5ppm) and Trp $C^{\beta}H_2$ (3.1ppm) protons in peptides **1** and **2**, respectively. Several NOEs are observed in the region 6.9-8.6 ppm, corresponding to peptide NH and Trp indole ring protons. Figure 1b demonstrates that irradiation of Trp $C^{\alpha}H$ in **1**, results in positive NOEs on the indole C2H and C4H protons, while a negative NOE is observed on the backbone Val NH proton. Irradiation of the Trp $C^{\beta}H_2$ resonance yields a strong positive NOE on the Trp C4H resonance, a weaker positive NOE on Trp C2H and a negative NOE on the Val NH proton. The additional negative NOEs observed in Figure 1 are due to partial saturation of the proximal $C^{\alpha}H$ and

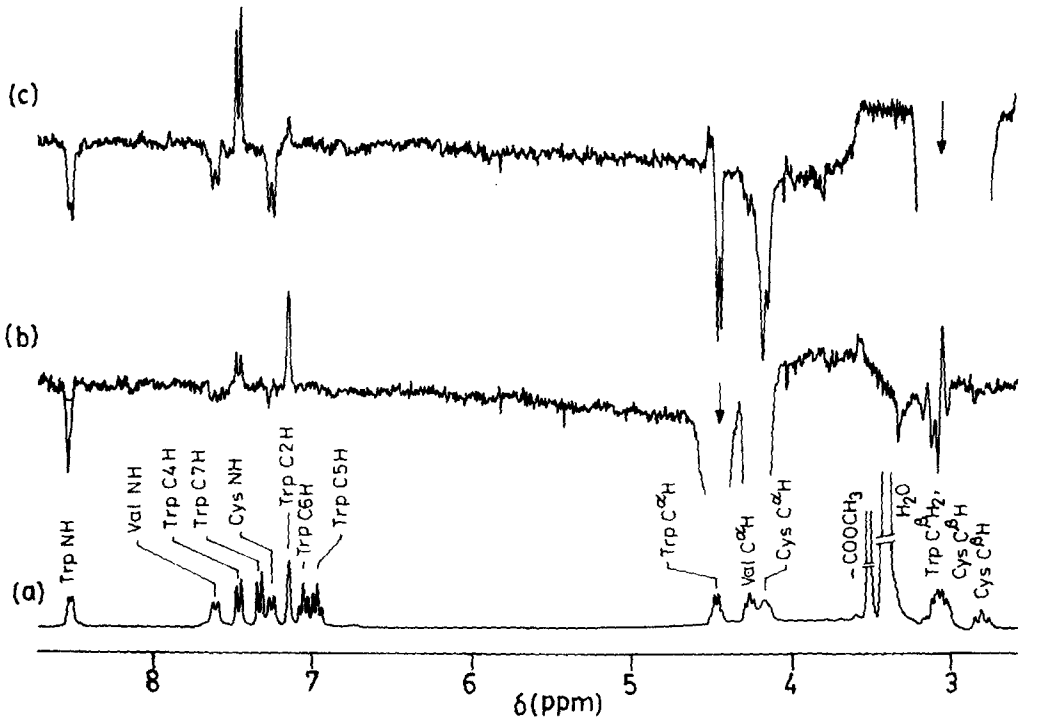

Figure 1. (a) Partial 270 MHz ^{1}H NMR spectrum of peptide **1** (n=1) in $(CD_3)_2SO$ at 293K. (b), (c) Difference NOE spectra obtained by saturating the Trp $C^{\alpha}H$ and $C^{\beta}H_2$ resonances, respectively. Points of irradiation are indicated by arrows. Difference spectra are magnified by a factor of 128.

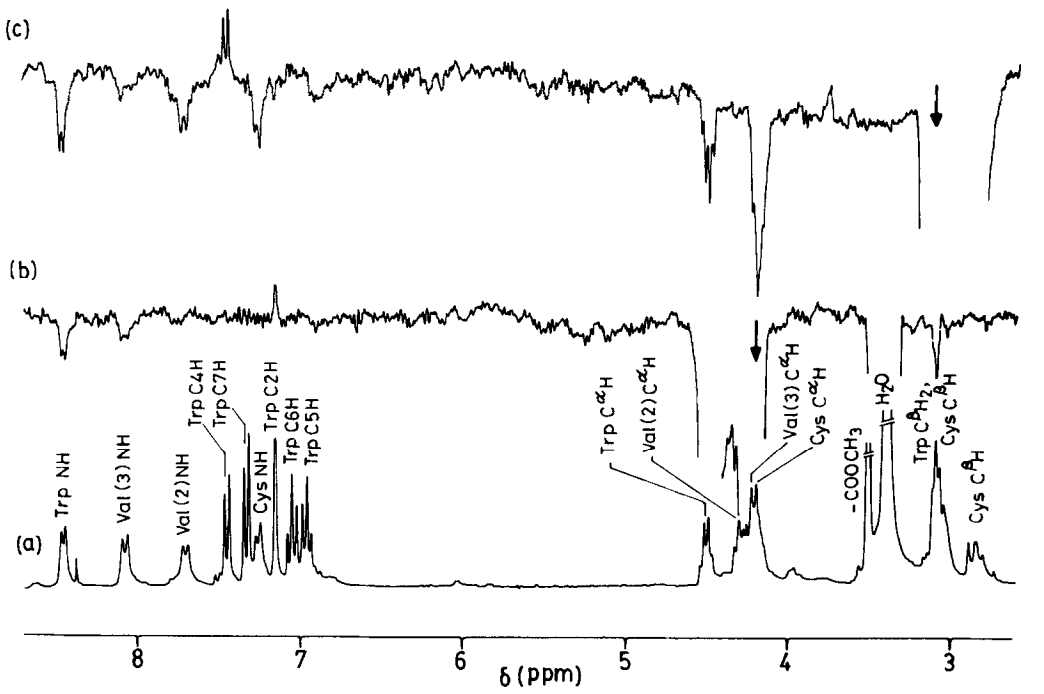

Figure 2. (a) Partial 270 MHz ^1H NMR spectrum of peptide **2** (n=2) in $(CD_3)_2SO$ at 293K. (b), (c) Difference NOE spectra obtained by saturating the Trp $C^{\alpha}H$ and $C^{\beta}H_2$ resonances, respectively. Arrows indicate points of irradiation. Difference spectra are magnified by a factor of 32.

$C^{\beta}H$ resonances. Similar observations are made for peptide **2** (Figure 2); although the magnitudes of the positive NOEs are diminished, the negative NOEs are enhanced. Table 1 summarizes specific NOE magnitudes determined for peptides **1-3.** Increasing molecular size diminishes the positive NOEs and enhances the negative NOEs. For **1** and **2**, experiments at high temperature (343K) establish that heating, which appreciably reduces solvent viscosity, enhances the value of the positive NOEs and indeed results in a change in the sign of the negative NOEs. The results suggest that these systems are characterized by $\omega\tau_c$ values of ~ 1 and as a consequence NOEs are markedly sensitive to changes in both molecular size and solvent viscosity. Furthermore, the observations are consistent with a situation where the rotational correlation time for molecular tumbling (τ_R) and the correlation time for segmental motion (τ_S) are not appreciably different. (The effective correlation time (τ_c) will be given by the equation $1/\tau_c = 1/\tau_R + 1/\tau_S$). Increasing molecular size also appears to impede segmental motion, presumably due to greater aggregation tendencies in the larger peptides.

TABLE 1

Selected NOEs observed in the Peptides Boc-Cys-(Val)$_n$-Trp-OMe on
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Boc-Cys-(Val)$_n$-Trp-OMe
Irradiation of Trp $C^{\alpha}H$ and Trp $C^{\beta}H_2$ resonances[a]

Resonance Irradiated	Trp $C^{\alpha}H$		Trp $C^{\beta}H_2$	
Peptide	Resonance Observed	NOE (%)	Resonance Observed	NOE (%)
1 (n=1)	Trp C2H	1.1 (3.0)	Trp C2H	0.3
	Trp C4H	0.7 (4.8)	Trp C4H	2.2
	Trp NH	-1.7 (1.9)	Trp NH	-1.6
			Trp $C^{\alpha}H$	-4.6
			Cys NH[b]	-1.7
			Cys $C^{\alpha}H$	-5.3
			Val(2)NH	-1.6
2 (n=2)	Trp C2H	0.5 (2.3)	Trp C2H	-0.5
	Trp C4H	0.0 (2.5)	Trp C4H	1.9
	Trp NH	-3.3 (1.1)	Trp NH	-3.7
	Val(3)NH[c]	-1.7	Trp $C^{\alpha}H$	-6.1
			Cys NH	-3.9
			Cys $C^{\alpha}H$	-7.0
			Val(2)NH	-3.9
3 (n=3)	Trp C2H	d	Trp C2H	e
	Trp C4H	d	Trp C4H	1.6
	Trp NH	-4.3	Trp NH	-7.2
	Val(4)NH[c]	-6.3	Trp $C^{\alpha}H$	-7.0
			Cys $C^{\alpha}H$	-9.0
			Val(2)NH	-5.3

[a] NOEs were measured in $(CD_3)_2SO$ at 293 K. Values in parentheses are NOEs observed on heating the sample to 343 K.

[b] NOEs seen on Cys $C^{\beta}H_2$, Cys NH and Val(2)NH are due to partial irradiation of Cys $C^{\alpha}H$.

[c] NOE is seen on Val(n)NH because of partial irradiation of Val $C^{\alpha}Hs$.

[d] No NOE could be detected. Under the experimental conditions used NOEs of 0.5% could be reliably observed.

[e] Trp C2H and Cys NH overlap.

Peptides **1-3** have been shown to favour antiparallel β-sheet conformations in $(CD_3)_2SO$ solutions (unpublished results), in accord with the tendency of cystine residues to stabilize such conformations by intramolecular hydrogen bonding involving the NH and CO groups of the residues preceding and succeeding cystine (6,7). The conformation depicted in Figure 3 permits rationalization of the observed NOEs. For dipole-dipole interactions involving the indole ring protons and the Trp $C^{\alpha}H$ and $C^{\beta}H_2$ protons, sidechain motions involving rotation about the C^{α} - C^{β} and C^{β} -C^{γ} bonds are important. For the backbone Trp $C^{\alpha}H \leftrightarrow$ Trp NH NOE or the other $C^{\alpha}_i H \leftrightarrow N_{i+1}H$ NOEs the reorientation

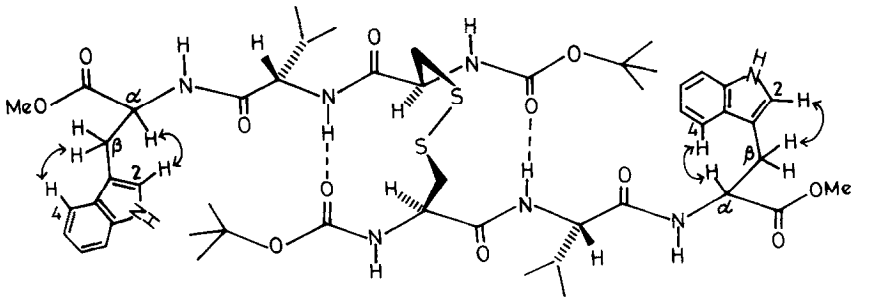

Figure 3. Antiparallel β-sheet conformation of peptide **1** (n=1). The Trp sidechains are schematically shown in two possible orientations about the $C^{\beta}-C^{\gamma}$ bond. Relevant NOEs for the sidechains are indicated by double edged arrows.

of the entire molecule is presumably important and negative NOEs result, since this is a slower process. The differences in the signs of the Trp $C^{\alpha}H \leftrightarrow C^{\beta}H$ and C2H(4H)$\leftrightarrow C^{\beta}H$ NOEs suggest that the dominant local motion is about the $C^{\beta}-C^{\gamma}$bond of Trp, which presumably corresponds to a rapid flipping motion of the indole ring (8). The simultaneous observation of the weak Trp $C^{\alpha}H \leftrightarrow$ Trp C4H and stronger Trp $C^{\alpha}H \leftrightarrow$ Trp C2H NOEs together with the observation of the weak Trp $C^{\beta}H \leftrightarrow$ Trp C2H and strong Trp $C^{\beta}H \leftrightarrow$ Trp C4H NOEs is indicative of differential population of various rotamers about the Trp $C^{\alpha}-C^{\beta}$ and $C^{\beta}-C^{\gamma}$ bonds. This is schematically indicated in Figure 3. The present studies demonstrate that NOEs can provide a rapid, qualitative assessment of the nature of segmental motion in oligopeptides.

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