MECHANISM OF THE INTRAMOLECULAR ^1H NUCLEAR OVERHAUSER EFFECT IN PEPTIDES AND DEPSIPEPTIDES [1]

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Summary: ^1H NMR double resonance studies of valinomycin in $(CD_3)_2SO$ were conducted at 90 MHz (FT-mode) and 250 MHz (correlation mode) to determine the mechanism of intramolecular nuclear Overhauser effects (NOE). These studies set specific constraints on any model for the conformation of valinomycin in $(CD_3)_2SO$ and illustrate that NOE experiments of this type hold considerable promise for conformational studies of peptides, proteins and other biomolecules. The NOE's are positive at the lower frequency and negative at the higher frequency. Consideration of the theoretical dependence of the NOE on the proton-proton internuclear correlation time and on the resonance frequency indicates that these results are explained by a predominantly dipolar relaxation mechanism.

Because the intramolecular NOE is a sensitive probe of spatial proximity of nuclei, it has yielded conformational information about a wide variety of small molecules (1). However, ^1H homonuclear NOE studies of peptide hormones and antibiotics have been limited, largely because the individual Overhauser enhancements for these relatively large molecules are small. Development of efficient methods for signal to noise enhancement by Fourier transform and correlation (2,3,4) techniques facilitates reliable detection of small intensity changes and makes possible the use of both intermolecular (5-9) and intramolecular (10,11) homonuclear NOE measurements in conformational studies of complex molecules. Recently Gibbons et al. reported ^1H NOE measurements at 220 MHz of the decapeptide antibiotic gramicidin-S in $(CD_3)_2SO$ (12). Negative Overhauser effects were observed

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Abbreviations: NOE, nuclear Overhauser effect; FID, free induction decay; Hyv, hydroxyvaleric acid; Lac, lactic acid; T_1, spin-lattice relaxation time.

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for spatially proximal nuclei on non-contiguous segments of the peptide backbone. This observation suggests that the intramolecular NOE holds great promise for investigating the three-dimensional structure of peptides.

If this type of Overhauser measurement is to be generally applicable to conformational studies of peptides, it is necessary that the mechanism responsible for the negative NOE be clearly elucidated. Toward this end we have conducted Overhauser measurements of the dodecadepsipeptide antibiotic valinomycin, (-L-Val-D-Hyv-D-Val-L-Lac-)$_3$, chosen because its NMR spectrum and solution conformation have been extensively investigated previously (13-21). The signs and magnitudes of the NOE's at 90 MHz and 250 MHz clearly indicate that the negative NOE at high magnetic field results from a dipolar relaxation mechanism in combination with a long internuclear correlation time.

MATERIALS AND METHODS

Sample concentrations are 4% (w/v) valinomycin (Sigma Chemical Co., St. Louis, Mo.) dissolved in $(CD_3)_2SO$ (Stohler Isotopes, Inc., Waltham, Mass.). NMR spectra were measured with 5mm sample tubes at probe temperatures of 29±1°C.; chemical shifts are referenced to the ^1H resonance of internal TMS.

^1H NMR spectra (90 MHz) were measured with a Bruker HX-90 operating in the pulse - Fourier transform mode. NOE's were determined by applying a 1.5 sec low power saturating pulse at the appropriate peak position, followed immediately by a high power 90° observing pulse. Since all the ^1H T_1's of valinomycin are less than 0.2 seconds, as determined by the inversion-recovery technique, the 1.5 sec presaturating pulse is long enough to allow Overhauser equilibrium to establish. Off-resonance control spectra were measured in the same manner except that the presaturating pulse was offset 1000 Hz to low field so that no solute resonances were perturbed. On-resonance and off-resonance spectra are the sums of 32 scans. Difference spectra were obtained by subtracting 256 off-resonance FID's from 256 on-resonance FID's followed by Fourier transformation.

At 250 MHz, spectra were acquired in the correlation mode (2,3) employing the Carnegie-Mellon superconducting spectrometer (4). NOE measurements were made with continuous saturation of the appropriate resonance. All spectra at this frequency (including those used in difference spectra) are the sum of 15 scans.

RESULTS AND DISCUSSION

Assignments of the ^1H NMR spectrum of valinomycin in $(CD_3)_2SO$ has been reported (13-17). Figure 1 illustrates typical NOE experiments with valinomycin at 90 MHz and 250 MHz. Saturation of the D-Val N$\underline{H}$ results in a positive NOE of 0.04 for the D-Hyv C$^{\alpha}\underline{H}$ resonance at 90 MHz; the same experiment at 250 MHz yields an NOE of -0.02 for the same proton. Intensity perturbations of peptide N$\underline{H}$ and C$^{\alpha}\underline{H}$ resonances, resulting from successive saturation of all other solute ^1H resonances at 90 MHz are summarized in Table 1. Although NOE measurements were not performed for all peaks at 250 MHz, the change in NOE sign from positive at 90 MHz to negative at 250 MHz was confirmed for several resonances.

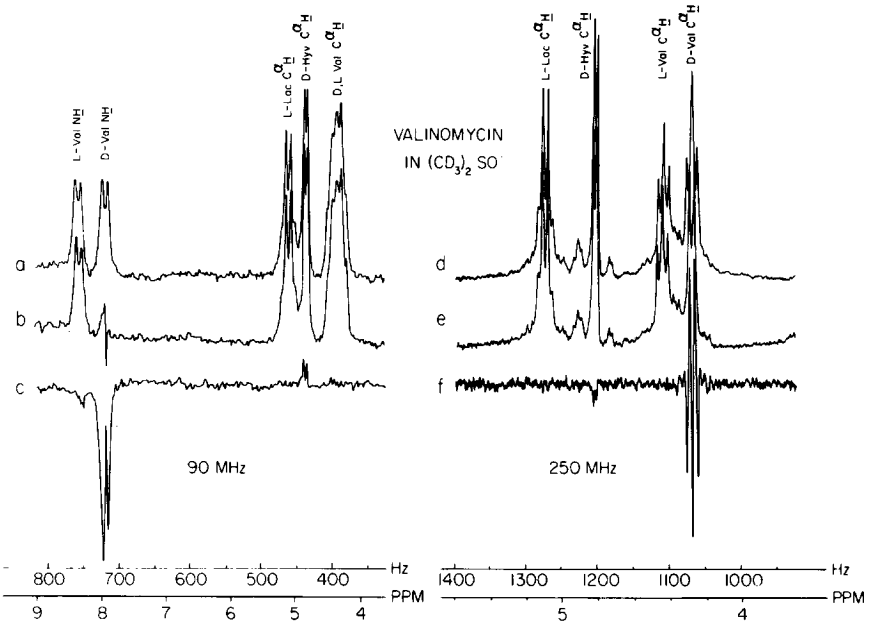

Figure 1. NOE measurements of valinomycin in $(CD_3)_2SO$ at 90 MHz by FT-NMR: a) control spectrum, b) spectrum obtained after low power presaturation of D-Val NH resonance, c) difference spectrum (spectrum b minus spectrum a multiplied by a factor of 2.0). NOE measurements at 250 MHz by correlation NMR: d) control spectrum (secondary rf 100 Hz to high field of D-Val NH resonance), e) spectrum obtained with simultaineous saturation of D-Val NH resonance, f) difference spectrum (spectrum e minus spectrum d multiplied by a factor of 3.1).

Negative homonuclear Overhauser effects can result from several mechanisms. Exchange modulation of the scalar coupling interaction (1) between saturated and observed nuclei always produces a decrease in resonance intensity. This mechanism does not appear to be operative for valinomycin, since it would not explain the observed positive NOE's at 90 MHz. Negative Overhauser effects are also predicted for dipolar interactions involving certain orientations of multispin systems described by Noggle and Schirmer (1). However, a positive NOE would not be predicted by this mechanism at 90 MHz.

For macromolecules a third mechanism must be considered: as molecular size increases and the correlation time (τ_c) between interacting spins increases, homonuclear NOE's become negative (22). Negative Overhauser effects have been observed for complexes of bovine neurophysin II with lysine vasopressin and various peptide analogs (5,6). The negative NOE's at 250 MHz for valinomycin can therefore be

Table 1. Nuclear Overhauser enhancements of peptide NH and CH protons of valinomycin in $(CD_3)_2SO$ at 90 MHz.

Resonance Saturated	Resonance Observed: L-Val NH	D-Val NH	L-Lac $C^{\alpha}H$	D-Hyv $C^{\alpha}H$	D,L-Val $C^{\alpha}H$
L-Val NH	-	...	0.06_8	0	0
D-Val NH	...	-	0	0.04_0	0
L-Lac $C^{\alpha}H$	0.05_8	0	-	...	...
D-Hyv $C^{\alpha}H$	0	0	...	-	...
D, L Val $C^{\alpha}H$	0	0	...	...	-
D,L Val, D-Hyv $C^{\beta}H^b$	0.04_6	0.04_6	0	0.05_4	0.09_9
Lac CH_3	0	0	0.09_0	0	0
Val, Hyv CH_3	<0.02	<0.02	0	0.07_6	0.06_5

a. The fractional increase in intensity (+10%) of the observed resonance upon saturation of specific resonances; (...) indicates peaks are too close to saturated resonance to be measured accurately.

b. Peaks overlap; cannot be distinguished.

explained readily in terms of a long rotational correlation time. The equations of Solomon (22) predict that for 7.1×10^{-10} sec $< \tau_c < 2.0 \times 10^{-9}$ sec the dipolar NOE is positive at 90 MHz and negative at 250 MHz. For $\tau_c < 7.1 \times 10^{-10}$ NOE's are positive at both frequencies (maximum NOE 0.5), whereas for $\tau_c > 2.0 \times 10^{-9}$ they are negative at both frequencies (minimum NOE -1) (1). Similar results have been obtained for ^{13}C-^{1}H (23,24) and ^{15}N-^{1}H interactions (25).

These NOE's place restrictions on any conformation proposed for valinomycin. Since NOE's are observed between the NH's of L-Val and $C^{\alpha}H$ of L-Lac, these protons must be in close proximity. Similar considerations apply for the NH protons of D-Val and $C^{\alpha}H$ protons of L-Lac. In contrast, no intra-residue NOE's are observed for the NH and $C^{\alpha}H$ of L and D-Val, suggesting that these protons are not proximal. Taken together these observations indicate, in agreement with earlier workers (13,16, 17,19,20) that the conformation of valinomycin is different in nonpolar solvents and highly polar solvents such as $(CD_3)_2SO$. In other words the "pore" and "core" (13,17) conformations observed in non-polar solvents are not retained in $(CD_3)_2SO$. In addition, these observations argue against a conformation in which the $C^{\alpha}H$-NH dihedral angles are nearly zero (13,17); such a conformation would juxtapose the $C^{\alpha}H$ and NH protons and would predict an Overhauser effect for the $C^{\alpha}H$ upon saturation of the peptide NH. These measurements illustrate that because of the dipolar origin of the NOE, this effect yields valuable information about the molecular geometry of peptides, depsipetides and related biomolecules. We are presently analyzing the Overhauser results and previous NMR data in detail to determine which, if any, of the proposed conformational models of valinomycin in $(CD_3)_2SO$ is the most suitable.

References

1. Noggle, J.H., and Shirmer, R.E. (1971) The Nuclear Overhauser Effect. (Academic Press, New York).

2. Dadok, J., and Sprecher, R.F. (1974) J. Magn. Res. 13, 243-248.

3. Gupta, R.K., Ferretti, J.A., and Becker, E.D. (1974) J. Magn. Res. 13, 275-290.

4. Dadok, J., Sprecher, R.F., Bothner-By, A.A., and Link, T. (1970), 11th Experimental NMR Conference, Pittsburgh, Pa.

5. Balaram, P., Bothner-By, A.A., and Dadok, J. (1972) J. Amer. Chem. Soc. 94, 4015-4017.

6. Balaram, P., Bothner-By, A.A., and Breslow, E. (1972) J. Amer. Chem. Soc. 94, 4017-4018.

7. Pitner, T.P., Glickson, J.D., and Dadok, J. (1974) Nature 250, 582-584.

8. Pitner, T.P., Glickson, J.D. Rowan, R., Dadok, J., and Bothner-By, A.A. (1975) J. Amer. Chem. Soc. 97, 5917-5918.

9. Glickson, J.D., Rowan, R., Pitner, T.P., Dadok, J., and Bothner-By, A.A. (1976) Biochemistry, in press.

10. Sykes, B.D., Weingarten, H.I., and Schlesinger, M.J. (1974) Proc. Nat. Acad. Sci. USA 71, 469-473.

11. Campbell, I.D., Dobson, C.M., and Williams, R.J.P. (1974) J. Chem. Soc. Chem. Comm. 888-889.

12. Gibbons, W. (1975) in Peptides: Chemistry, Structure and Biology, eds. Walter, R. and Meienhofer, J. (Ann Arbor Science Publishers, Ann Arbor, Michigan), pp. 127-138.

13. Ohnishi, M., and Urry, D.W. (1969) Biochem. Biophys. Res. Commun. 36, 194-202.

14. Ivanov, V.T., Laine, I.A., Abdulaev, L.B., Senyavina, L.B., Popov, E.M., Ovchinikov, Yu, A., and Shemyakin, M.M. (1969) Biochem. Biophys. Res. Commun. 34, 803-811.

15. Haynes, C.C., Kowalsky, A., and Pressman, B.C. (1969) J. Biol. Chem. 244, 502-505.

16. Ovchinikov, Yu, A., Ivanov, V.T., Evstratov, A.V., Laine, I.A. (1971) in Peptides 1969, ed. Scoffone, E. (North-Holland Publishing Company, Amsterdam), pp. 266-281.

17. Urry, D.W., and Ohnishi, M. (1970) in Spectroscopic Approaches to Biomolecular Conformation, ed. Urry, D.W. (American Medical Association Press, Chicago, Illinois), chapter 7.

18. Funck, T., and Grell, E. (1973) Eur. J. Biochem. 34, 415-424.

19. Patel, D.J., and Tonelli, A.E. (1973) Biochemistry 12, 486-496.

20. Patel, D.J. (1973) Biochemistry 12, 496-501.

21. Urry, D.W., and Kumar, N.G. (1974) Biochemistry 13, 1829-1831.

22. Solomon, I. (1955) Phys. Rev. 99, 559-565.

23. Allerhand, A., and Oldfield, E. (1973) Biochemistry 12, 3428-3433.

24. Allerhand, A., Childers, R.F., and Oldfield, E., (1973) Ann. N.Y. Acad. Sci. 222, 764-777.

25. Hawkes, G.E., Litchman, W.M., and Randall, E.W. (1975) J. Magn. Reson. 19, 255-258.