NUCLEAR OVERHAUSER ENHANCEMENT DEMONSTRATION OF THE TYPE II β-TURN IN REPEAT PEPTIDES OF TROPOELASTIN

Md. Abu Khaled and Dan W. Urry

Laboratory of Molecular Biophysics
and the
Cardiovascular Research and Training Center
University of Alabama Medical Center
Birmingham, Alabama 35294

Received March 15,1976

SUMMARY: Nuclear Overhauser enhancement (NOE) experiments have been performed with the elastin peptides, namely; HCO-Val_1-Pro_2-Gly_3-Gly_4-OMe, t-Boc-Val_1-Pro_2-Gly_3-Val_4-Gly_5-OMe and t-Boc-Val_6-Ala_1-Pro_2-Gly_3-Val_4-Gly_5-OMe in DMSO-d_6. An NOE of approximately 10% was observed between the αCH of Pro_2 and the NH of Gly_3 involved in the β-turn of all three peptides. This finding shows the close proximity of two aforementioned protons and thus shows the occurrence of Type II β-turn in the repeat elastin peptides. The intermolecular distances are calculated and compared with the distances obtained from other model systems.

INTRODUCTION

Currently, the investigation of the occurrence of β-turns in peptides is becoming important in the field of peptide and protein research. From an extensive study on peptides by NMR (1,2) and IR (3) the formation of a β-turn has been shown to occur in peptides by the stabilization of a 1 ← 4 type H-bond. In repeat peptides of elastin, namely; Val_1-Pro_2-Gly_3-Gly_4 (tetra), Val_1-Pro_2-Gly_3-Val_4-Gly_5 (penta) and Ala_1-Pro_2-Gly_3-Val_4-Gly_5-Val_6 (hexa) the formation of β-turns has been shown to occur (1) with Pro_2 and Gly_3 at the corners of this 10-membered ring and a Type II was assumed from the qualitative conformational energy considerations (1). Such a β-turn in a linear peptide can occur in two forms (4) which are Type I (Figure 1A) and Type II (Figure 1B). The difference between these two types

of β-turns is an approximate 180^{o} rotation of the peptide moiety between Pro_2 and Gly_3 (Figure 1). Theoretical calculations (5) on pentapeptide (VPGVG) have argued for the occurrence of a Type II β-turn (Figure 1B). It can be noticed in Figure 1 that in Type II the α proton of Pro_2 is in close proximity to the peptide proton of Gly_3 whereas in Type I they are further apart.

Nuclear Overhauser effect (NOE) is now in wide-spread use in structural organic chemistry (6). A recent application of this method has been to show the close, through-space proximity of protons in tyrocidine A (7). If a Type II β-turn occurs in the repeat peptides of elastin, then NOE is expected to be observed between the αCH proton of Pro_2 and NH proton of Gly_3. Two Type I β-turns can be considered to occur in the valinomycin-K^+ complex involving D-HyIV and D-Val in one ring and L-Lac and L-Val in another. The crystal structure data (8) gives a 3.4 Å distance between the αCH proton of L-Lac and the NH proton of L-Val. A correlation between the observed NOE and the internuclear distance (r_{xy}) between two protons or group of protons, x and y is given by the expression (9)

$$NOE = 1/Kr_{xy}^{6} \qquad (1)$$

where K is a constant. Use of this equation once K is evaluated on a model system, gives an approximate internuclear distance between the protons which show an enhancement of signal intensity. Therefore, NOE experiments can be used to identify experimentally the type of β-turn in the repeat peptides of elastin in solution, once validated on the valinomycin-K^+ complex.

MATERIALS AND METHODS

The repeat peptides of elastin, HCO-Val_1-Pro_2-Gly_3-Gly_4-OMe (VPGG), t-Boc-Val_1-Pro_2-Gly_3-Val_4-Gly_5-OMe (VPGVG) and t-Boc-Val_6-Ala_1-Pro_2-Gly_3-Val_4-Gly_5-OMe (VAPGVG) were synthesized in

this Laboratory (10-12). Solutions (0.1M) of each of these peptides in DMSO-d_6 (99.9%) and of the valinomycin-K^+ complex in $CDCl_3$ (99.8%) were made. The samples were degassed several times by the freeze-thaw method and sealed in an NMR tube under vacuum.

NMR experiments were performed on a JEOL PS-100 NMR spectrometer operating at a probe temperature of 22°C and in the internal lock mode. The enhancement of signal intensity was measured from the difference of the integrated signal areas (at least five times) before and after saturation of the signal of interest. As the level of the second field (f_2), which is used for the double resonance experiments (13), also affects the areas of all signals in the spectrum, the signal area was integrated when f_2 was saturating the signal of interest and then compared with the signal area obtained when the same level of f_2 was offset to a region of the spectrum containing no absorption signal.

RESULTS

The spectral regions of concern (at 100 MHz) for NF-Val_1-Pro_2-Gly_3-Gly_4-OMe (tetra), t-Boc-Val_1-Pro_2-Gly_3-Val_4-Gly_5-OMe (penta) and of t-Boc-Val_6-Ala_1-Pro_2-Gly_3-Val_4-Gly_5-OMe (hexa) obtained in DMSO-d_6 are shown in Figure 2. The spectra are expanded to a suitable width for measuring signal enhancements. The signal assignments are made according to the previous studies (10-12). It can be seen in Figure 2A that the signal intensity of the Gly_3 NH proton of NF-Val_1-Pro_2-Gly_3-Gly_4-OMe is increased when the $\alpha C\underline{H}$ of Pro_2 is saturated. Similarly, the intensity of the Pro_2 $\alpha C\underline{H}$ is increased when the NH of Gly_3 is saturated (see Figure 2B) and the percentage of enhancement (10.2%, see Table I) is the same in both the cases. In the same way, on saturating the $\alpha C\underline{H}$ of Pro_2 in t-Boc-Val_1-Pro_2-Gly_3-Val_4-Gly_5-OMe, an enhance-

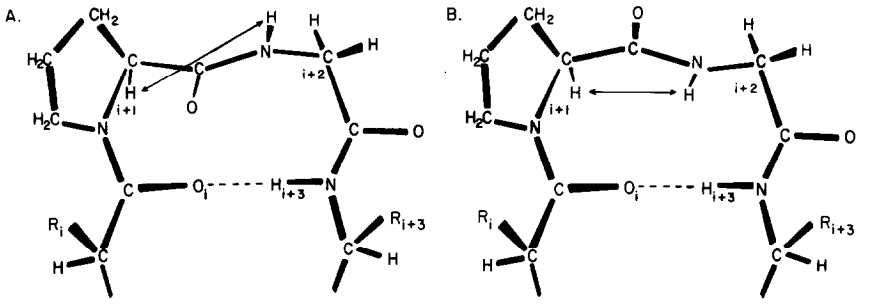

Fig. 1. β-Turns in peptides.

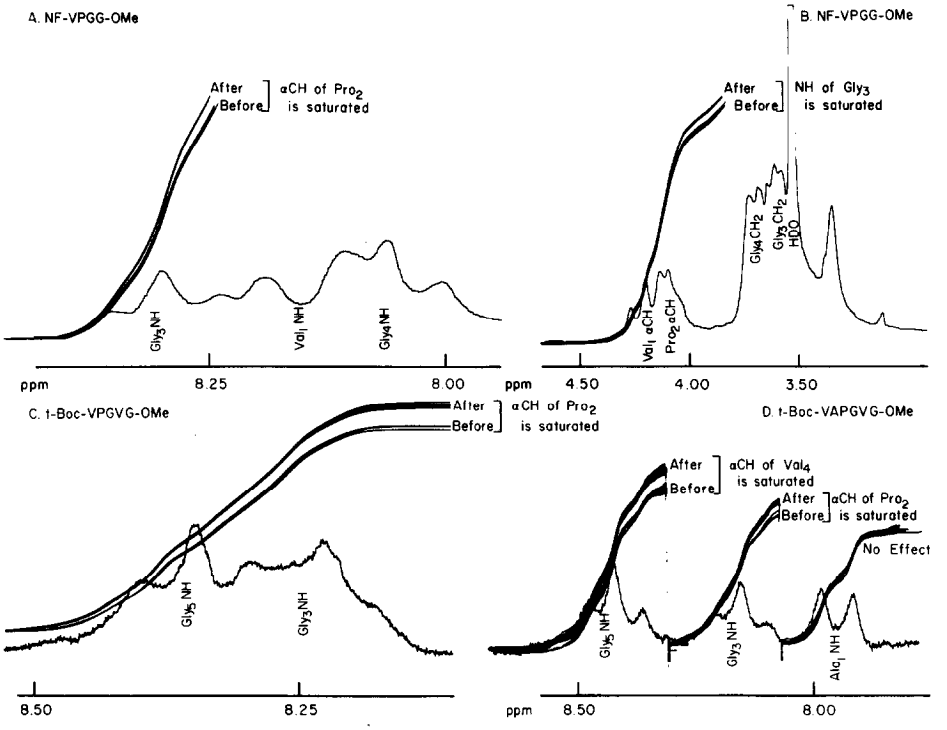

Fig. 2. Spectra obtained at 100 MHz in DMSO-d_6.

ment of the Gly_3 NH signal intensity is observed (see Figure 2C) and on saturating the αCH of Pro_2 of t-Boc-Val_6-Ala_1-Pro_2-Gly_3-Val_4-Gly_5-OMe a signal enhancement of Gly_3 NH is observed (see Figure 2D). It can be noticed that a considerable enhancement of the signal intensity of the Gly_5 NH in the penta and the hexa

TABLE I

COMPOUNDS	SOLVENTS	NOE IN %	DISTANCE (Å) CALCULATED USING EQUATION 1	DISTANCE (Å) FROM MODEL SYSTEMS
Tetra	DMSO-d_6	10.2 ± 0.5	2.86	2.5[a,b]
Penta	DMSO-d_6	9.5 ± 1.0	2.89	2.4[c]
Hexa	DMSO-d_6	9.8 ± 1.5	2.87	2.7[a]
VMK$^+$	$CDCl_3$	1.9 ± 1.5	3.78	3.4[d]

a. An approximate distance measured from the Dreiding model.
b. From theoretical calculation (V. Renugopalakrishnan, private communication).
c. See Reference 5.
d. See Reference 8.

peptides is also obtained (see Figures 2C and 2D). This arises due to the formation of an 11-membered ring in these compounds (14-16) which will be discussed in detail in a subsequent publication on the analysis of the glycine coupling constants of the repeat peptides of elastin. As a control, an experiment was performed with the valinomycin-K^+ complex in $CDCl_3$ in an effort to assess the NOE on the resonance intensity of the αCH of L-Lac on saturation of the NH of L-Val. Only limited enhancement is obtained which is barely greater than the experimental error (see Table I). Using Equation 1 and $K = 1.8 \times 10^{-2}$ (see Reference 9), an approximate internuclear distance is calculated and the results are given in Table I.

DISCUSSION

It is well-established that a β-turn is formed in these repeat peptides of elastin (10-12), two possible structures of

which are given in Figure 1. The distances between the αCH Pro and NH Gly, obtained from Dreiding models of the β-turn and from the theoretical calculation, are given in Table I. There are small differences in the distances obtained by NOE, from the model building and from the theoretical calculations. This is because in solution average conformations are observed for these molecules and, accordingly, a slightly greater average experimental distance is expected. Clearly the NOE experiments show the close proximity of the αCH Pro and NH Gly protons and thereby are consistent with the occurrence of a Type II β-turn (Figure 1B) in these peptides of elastin.

Given a distance of 3.4 Å between the αCH of L-Lac and the NH of L-Val in the valinomycin-K^+ (8), one calculates by Equation 1 an expected NOE of 3.5%. This is a value closely within the experimental error of the measurement for a rigid model system containing two protons with a through-space distance equivalent to that of a Type I β-turn. The absence of a significant NOE for this system (see Table I) substantiates the absence of an NOE for a Type I β-turn. Therefore, the above results provide the first direct experimental demonstration of Type II β-turns in the repeat peptides of elastin.

ACKNOWLEDGMENT: This work was supported by the National Institutes of Helath Grant No. HL-11310.

REFERENCES:

1. Urry, D. W., and Long, M. M. (1976) CRC Crit. Rev. Biochem. (in press).
2. Kopple, K. D., and Go, A. (1975) Peptides: Chemistry, Structure and Biology (Walter, R. and Meienhofer, J., eds.) p. 139-150, Ann Arbor Scientific Publishing, Ann Arbor, Michigan.
3. Bounnard, G., Marraud, M., and Neel, J. (1974) J. Chim. Phys. 71, 1081-1091.
4. Venkatachalam, C. M. (1968) Biopolymers 6, 1425-1436.

5. Renugopalakrishnan, V., and Urry, D. W. (1976) Int. J. Quantum Chem.:QBS No. 3 (in press).
6. Noggle, J. H., and Schirmer, R. E. (1971) The Nuclear Overhauser Effect, Academic Press, New York.
7. Gibbons, W. A., Beyer, C. F., Dadok, J., Sprecher, R. F., and Wyssbrod, H. R. (1975) Biochemistry 14, 420-429.
8. Neupert-Laves, K., and Dobler, M. (1975) Helv. Chim. Acta 58, 432-442.
9. Bell, R. A., and Saunders, J. K. (1970) Can. J. Chem. 48, 1114-1122.
10. Urry, D. W., and Ohnishi, T. (1974) Biopolymers 13, 1223-1242.
11. Urry, D. W., Cunningham, W. D., and Ohnishi, T. (1974) Biochemistry 13, 609-615.
12. Urry, D. W., and Ohnishi, T. (1974) Peptides, Polypeptides and Proteins (Bovey, F. A., Goodman, M. and Lotan, N., eds.) p. 230-247, John Wiley and Sons, Inc., New York.
13. von Philipsborn, W. (1971) Angew. Chem. 10, 472-490.
14. Urry, D. W., Mitchell, L. W., and Ohnishi, T. (1975) Biochim. Biophys. Acta 393, 296-306.
15. Urry, D. W., Ohnishi, T., Long, M. M., and Mitchell, L. W. (1975) Int. J. Pept. Protein Res. 7, 367-378.
16. Urry, D. W., Mitchell, L. W., Ohnishi, T., and Long, M. M. (1975) J. Mol. Biol. 96, 101-117.