NUCLEAR OVERHAUSER ENHANCEMENT EVIDENCE FOR INVERSE TEMPERATURE DEPENDENCE OF HYDROPHOBIC SIDE CHAIN PROXIMITY IN THE POLYTETRAPEPTIDE OF TROPOELASTIN

D. W. Urry, M. A. Khaled, R. S. Rapaka and K. Okamoto

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

Received September 23,1977

SUMMARY:
Previous studies on aqueous solutions of HCO-(Val-Pro-Gly-Gly)$_{40}$-Val-OMe indicated an increase in secondary structure on increasing the temperature implying a concomitant intramolecular hydrophobic association. Nuclear Overhauser enhancement (NOE) studies are reported which explicitly demonstrate an increase in association of γCH_3 of Val and δCH_2 of Pro protons on increasing temperature. The analogue where Ala replaces Val does not show this inverse temperature transition. These results provide direct demonstration of the "hydrophobic effect" responsible for inverse temperature transitions in aqueous systems.

INTRODUCTION:

Previous studies on the structure of elastin peptides in water have suggested the occurrence of an inverse temperature transition in which, on increasing the temperature, the structure becomes more ordered both intermolecularly and intramolecularly (1-3). The driving force for this increase in order of the peptide portion of the system is explicable as being an increase in entropy of more ordered water surrounding exposed hydrophobic side chains which becomes less ordered bulk water as the hydrophobic side chains associate (4,5). The intermolecular increase in order on increasing temperature is observed for the elastin peptides wherein molecules randomly distributed in solution begin, on raising the temperature to coalesce to form aggregates which are seen as regular filamentous arrays, when observed in electron micrographs of negatively stained specimens (6-10). This reversible temperature ellicited coalescence is called coacervation. The intramolecular increase in

ISSN 0006-291X

order is observed by circular dichroism (11,12) and is implied by nuclear magnetic resonance studies which indicate an increase in shielding from solvent of specific N-H and C-O moieties on increasing the temperature (13,14).

In particular with the repeat peptides of tropoelastin -- HCO-$(Val_1$-Pro_2-Gly_3-$Gly_4)_n$-Val-OMe and HCO-$(Val_1$-Pro_2-Gly_3-Val_4-$Gly_5)_n$-Val-OMe -- there is observed in several polar organic solvents and at low temperature in water, a β-turn, with Pro_2 and Gly_3 at the corners and with the Val_1 C-O···H-N residue-4 10-atom hydrogen bonded ring. On increasing the temperature in water the Val_1 NH becomes shielded from the solvent and this shielding has been correlated with increase in shielding of the residue-4 C-O. This suggests the presence of a 14-atom hydrogen bonded ring (13). Adding the 14-atom hydrogen bonded ring to the β-turn on raising the temperature of aqueous solutions of the polytetrapeptide and the polypentapeptide places Val_1 and Pro_2 side chains in juxtaposition. The β-turn and 14-atom hydrogen bonded ring are also observed for the monomers of the tetrapeptide and the pentapeptide in the non-polar solvent chloroform and in <u>in vacuo</u> conformational energy calculations (15,16). In the repeat hexapeptide of tropoelastin $(Ala_1$-Pro_2-Gly_3-Val_4-Gly_5-$Val_6)_n$ residue-1 is a less bulky and less hydrophobic alanine residue with the result that the 14-atom hydrogen bonded ring does not form (13). Therefore it is anticipated that the specific intramolecular hydrophobic association, responsible for the inverse temperature transition observed in water with the polytetrapeptide and polypentapeptide, is the Val_1-Pro_2 side chain interaction. Accordingly we have looked in HCO-$(Val_1$-Pro_2-Gly_3-$Gly_4)_n$-Val-OMe for a temperature dependent nuclear Overhauser enhancement (NOE) between the Val_1 $\gamma C\underline{H}_3$ protons and the δCH_2 protons of Pro_2 and as a control have examined HCO-$(Ala_1$-Pro_2-Gly_3-$Gly_4)_n$-Val-OMe for possible temperature dependence of NOE between the $C\underline{H}_3$ of Ala_1 and the $\delta C\underline{H}_2$ of Pro_2. This control is particularly pertinent as HCO-$(Val_1$-Pro_2-Gly_3-$Gly_4)_n$-Val-OMe shows the intramolecular inverse temperature transition and when n is large enough coacervates whereas HCO-$(Ala_1$-Pro_2-Gly_3-$Gly_4)_n$-Val-OMe does not.

MATERIALS AND METHODS:

Experimental

The tetrapeptides, H-(L-Val_1-L-Pro_2-Gly_3-Gly_4)$_n$-Val-OMe, $(VPGG)_n$, and H-(L-Ala_1-L-Pro_2-Gly_3-Gly_4)$_n$-Val-OMe, $(APGG)_n$, where $n \geq 40$, were synthesized in this laboratory (17). A solution 3-5% (w/v) of each of these peptides was made in D_2O and purged with argon gas for at least 15 minutes. Each of these solutions was carefully degassed in the PMR tube by freeze-thawing and then sealed under vacuum. This sample preparation is necessary when performing NOE experiments in order to remove dissolved oxygen from the aqueous solutions.

NOE experiments were performed on a JEOL PS-100 spectrometer operating in the internal lock mode and equipped with a JEOL JNM VT-3B temperature controller. The signal enhancement (NOE) was measured from the difference of the integrated signal areas, more than five times (see Figure 1), with and without the second field (f_2) at the resonance frequency of the signal of interest. Since a degassed sample has $T_1 \cdot T_2 > 10$, which delays the recovery of the magnetization (18), it was necessary to allow sufficient time (at least one minute) between successive integrations of signal area. As the level of the second field (f_2) also affects the observed intensities of all signals in the spectrum, the signal area was integrated when f_2 was at the signal of interest and then compared with the area obtained when the same level of f_2 was offset to a region of the spectrum which contained no absorption signal.

The temperatures used for the study were 20°C and 40°C. The 40°C high temperature value was chosen because $(VPGG)_n$ begins to associate at 45°C to form the more viscous coacervate solution.

RESULTS AND DISCUSSION

Nuclear Overhauser enhancement has been utilized as a method with which to observe proton-proton proximity in organic molecules (18-20). The relationship between % NOE and proton proximity has an inverse sixth power dependence such that only when a pair of protons or groups of equivalent protons occur for

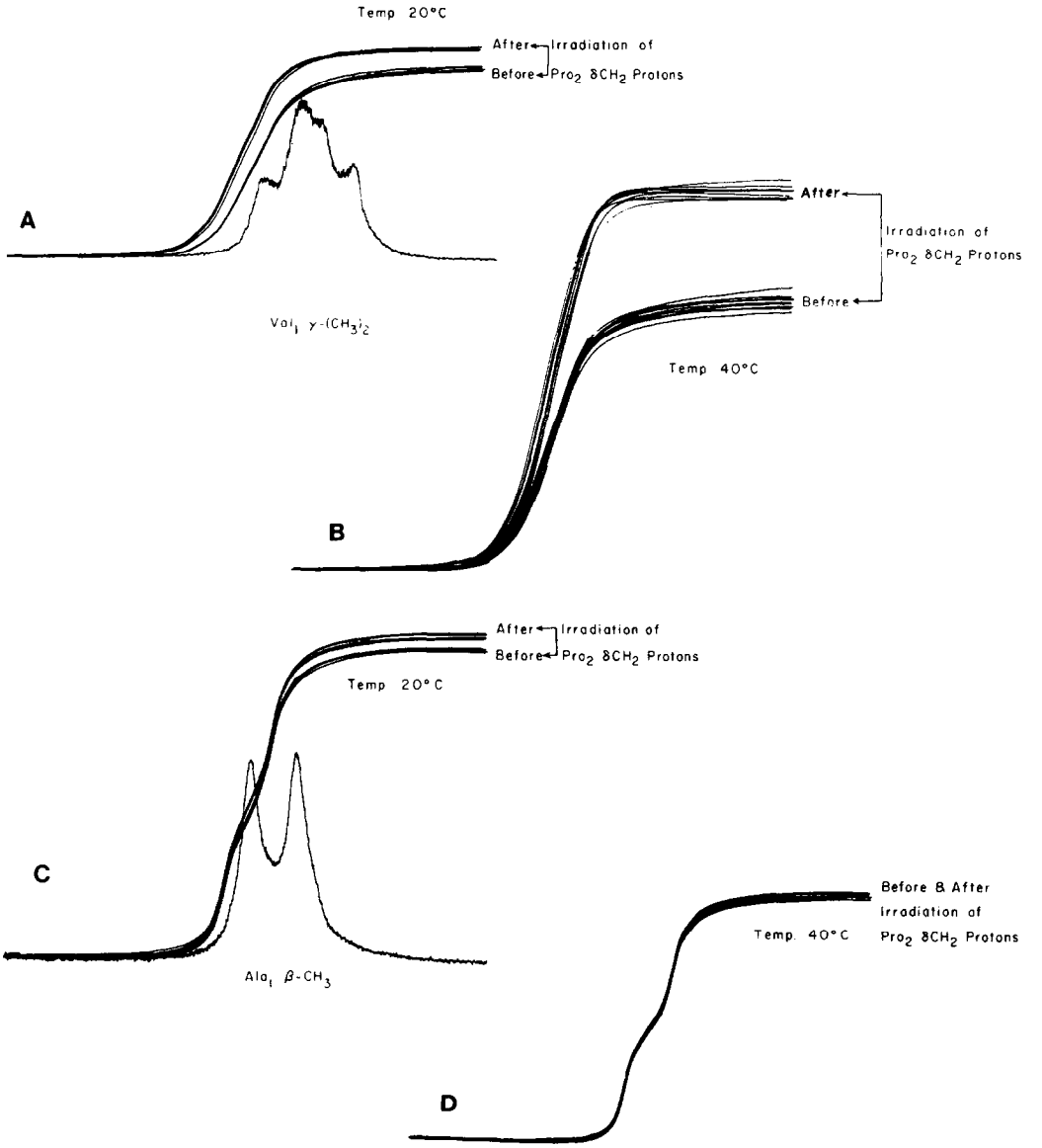

Figure 1: Signal enhancement on Val_1 γ-$(CH_3)_2$ protons (NOE) by irradiating Pro_2 δ-CH_2 protons in H-(L-Val_1-L-Pro_2-Gly_3-Gly_4)$_n$-Val-OMe in D_2O: A. At a temperature of 20°C and B. At a temperature of 40°C. NOE of Ala β-CH_3 protons on irradiation of Pro_2 δ-CH_2 protons in H-(L-Ala_1-L-Pro_2-Gly_3-Gly_4)$_n$-Val-OMe in D_2O: C. At a temperature of 20°C. D. At a temperature of 40°C.

significant periods of time at distances closer than 3 Å are measurable values for % NOE obtained. In the present experiments the question of concern is the temperature dependence of the relative proximity in $(APGG)_n$ of the $\delta C\underline{H}_2$ protons of the Pro_2 residue to the β-$C\underline{H}_3$ protons of Ala_1 and in $(VPGG)_n$ of the proximity of the $\delta C\underline{H}_2$ protons of the Pro_2 residue to the $\gamma C\underline{H}_3$ protons of Val_1.

At 20°C irradiation of the $\delta C\underline{H}_2$ of $(APGG)_n$ results in a 4% enhancement of the β-$C\underline{H}_3$ proton intensity (see Figure 1c), whereas on increasing the temperature to 40°C, there is no detectable increase in signal intensity (see Figure 1d). The small NOE is no longer observed in $(APGG)_n$ on raising the temperature. On the other hand irradiation of the $\delta C\underline{H}_2$ protons of $(VPGG)_n$ at 20°C results in a larger, 14% enhancement of the γCH_3 proton signal intensity and, strikingly, on raising the temperature to 40°C there is a dramatic increase of $\gamma C\underline{H}_3$ signal intensity to result in a 43% enhancement (see Figures 1a and 1b). In contrast to $(APGG)_n$, raising the temperature of aqueous solutions of $(VPGG)_n$ results in increased proximity of $C\underline{H}_3$ and $\delta C\underline{H}_2$ protons.

As noted above this valyl prolyl hydrophobic side chain association was previously deduced in aqueous solution on the basis of an inferred increase in secondary structure with increase in temperature (see Reference 13) when it was shown that on raising the temperature both the Val_1 $N\underline{H}$ and the residue-4 C-O became more shielded from solvent as occurs on formation of a hydrogen bond. Such an inverse temperature transition requires consideration of the aqueous solvent. The formation of a Val_1 NH residue-4 C-O hydrogen bond fixes the ϕ_1 and ψ_1 dihedral angles such that the Val_1 and Pro_2 side chains become juxtaposed (see Figure 2). It was, therefore, considered implicit that the inverse temperature transition involved a decrease in the amount of ordered water surrounding valyl and prolyl side chains thereby allowing the side chains to come into direct contact (For a schematic representation see Ref. 1, Figure 2). The present data explicitly demonstrate the hydrophobic side chain association attending an inverse temperature transition in an aqueous system and provide direct evidence for what has been loosely termed "hydrophobic

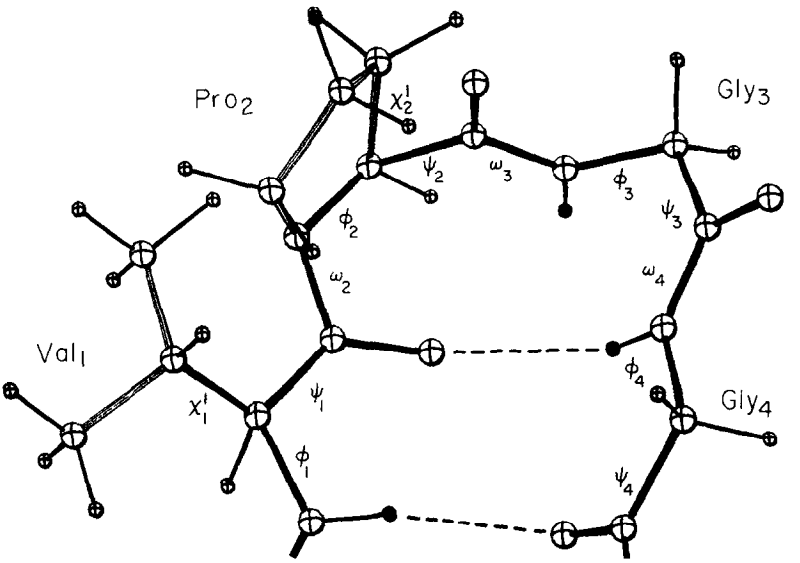

Figure 2: Proposed conformation of the tetrapeptide, Val_1-Pro_2-Gly_3-Gly_4, derived from nuclear magnetic resonance studies in chloroform and from conformational energy calculations (reproduced with permission from Reference 15). The same conformation had been previously proposed for the repeating unit of the polytetrapeptide in water at elevated temperature (13). It is included here to facilitate visualization of the relationship between formation of the 14-atom hydrogen bonded ring and the Val_1 and Pro_2 side chain association.

bonding." Such hydrophobic associations are of primary importance in numerous macromolecular assemblages within biological organisms.

A more detailed treatment of the polytetrapeptide results and inclusion of the polypentapeptide results are in preparation.

ACKNOWLEDGMENT: This work was supported in part by the National Institutes of Health, Grant No. HL-11310.

REFERENCES:

1. Urry, D. W. (1976) Faraday Disc. Chem. Soc., No. 61, 205-212.
2. Urry, D. W., Long, M. M., and Mitchell, L. W. (1976) Int. J. Quantum Chem.: Quantum Biology Symp. No. 3, 107-118

3. Urry, D. W. (1977) Perspect. Biol. Med. (in press).
4. Kauzmann, W. (1959) Adv. Protein Chem., 14, 1.
5. Tanford, C. (1973) "The Hydrophobic Effect: Formation of Micelles in Biological Membranes," John Wiley and Sons, New York.
6. Cox, B. A., Starcher, B. C., and Urry, D. W. (1973) Biochim. Biophys. Acta, 317, 209-213.
7. Cox, B. A., Starcher, B. C., and Urry, D. W. (1974) J. Biol. Chem., 249, 997-998.
8. Urry, D. W., Long, M. M., Cox, B. A., Ohnishi, T., Mitchell, L. W., and Jacobs, M. (1974) Biochim. Biophys. Acta, 371, 597-602.
9. Volpin, D., Urry, D. W., Pasquali-Ronchetti, I., and Gotte, L. (1976) Micron, 7, 193-198.
10. Volpin, D., Urry, D. W., Cox, B. A., and Gotte, L. (1976) Biochim. Biophys. Acta, 439, 253-258.
11. Urry, D. W., Starcher, B., and Partridge, S. M. (1969) Nature, 222, 795-796.
12. Starcher, B. C., Saccomani, G., and Urry, D. W. (1973) Biochim. Biophys. Acta, 310, 481-486.
13. Urry, D. W., and Long, M. M. (1976) CRC Crit. Rev. Biochem., 4, 1-45.
14. Urry, D. W., and Long, M. M. (1977) Adv. Exp. Med. Biol., 79, 685.
15. Khaled, Md. Abu, Renugopalakrishnan, V., and Urry, D. W. (1976) J. Am. Chem. Soc., 98, 7547-7553.
16. Renugopalakrishnan, V., Khaled, Md. Abu, and Urry, D. W. (1977) J. Chem. Soc. (in press).
17. Rapaka, R. S., Okamoto, Kouji, and Urry, D. W. (in preparation).
18. Noggle, J. H., and Schirmer, R. E. (1971) The Nuclear Overhauser Effect, Chemical Application., Acad. Press., New York and London.
19. Gibbons, W. A., Crepann, D., Delyre, J., Dunand, J., Hajdnkovic, G., and Wyssbrod, H. A. (1975) "Peptides: Chemistry, Structure and Biology," Walter, R., and Meinhofer, J., Eds., Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, p. 127-136.
20. Khaled, M. A., and Urry, D. W. (1976) Biochem. Biophys. Res. Commun., 70, 485-491.