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ON THE CONFORMATION OF LUTEINIZING HORMONE-RELEASING HORMONE, NUCLEAR OVERHAUSER OBSERVATIONS*

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Summary: Observations of proton nuclear Overhauser effects in the molecule Luteinizing Hormone-Releasing Hormone indicate that a high population of a particular set of conformers exists in water solution. The results can be interpreted as two distinct conformers in which the $pGlu^1$ ring is in close proximity to aromatic residues further along the sequence of the linear structure. The observed nuclear Overhauser effects were in agreement with the enhancements calculated from models obtained by conformational energy calculations.

Recent conformational energy calculations (1-3) on the molecule LHRH have resulted in the prediction of two low energy conformations which appear to explain much of the experimental analog data (2,3). Previous studies by NMR (4-7), ORD/CD (8), and molecular luminesence (9,10), which have given little information about the conformation of LHRH in solution, have led workers to suggest that LHRH appears to behave as a random coil polypeptide and is devoid of any intrachain residue-residue interactions in solution (8).

In order to test the possibility that the most stable conformation in solution is that found by conformational energy calculations, we have carried out a proton nuclear Overhauser study of LHRH at 300 MHz. The results of our initial experiments are reported here.

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Abbreviations: LHRH, Luteinizing Hormone-Releasing Hormone; pGlu, pyroglutamic acid; NOE, nuclear Overhauser effect

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MATERIALS AND METHODS

The sample was prepared by distilling deuterium oxide (99.5%d) under high vacuum onto 9.4mg of LHRH which had been lyophilized against deuterium oxide and dried overnight under high vacuum. The solution was prepared in the inner tube of a Wilmad coaxial cell and sealed off under high vacuum. The sample contained approximately 0.25ml of deuterium oxide. A lock signal was provided by trifluoroacetic acid in the outer annulus of the cell. The NMR spectrometer was a modified Varian HR300 spectrometer and was operated in correlation mode (11) with the aid of a Varian 620-f100 computer. The saturating field was provided by a computer controlled synthesizer (Rockland Model 5100) and difference spectra were computed from scans taken with the synthesizer alternately on and off resonance. The effective saturating field was calibrated in the usual way (12) and was found to be near 2mG. Chemical shift assignments were made on the basis of the work of Wessels et. al. (6) and verified by double resonance experiments where required.

RESULTS AND DISCUSSION

Saturation of the resonance due to the $pGlu^{1}C\beta,\gamma$ and $Pro^{9}C\beta$ protons resulted in decreases in the amplitudes of several of the aromatic resonances. The largest decrease observed was in the resonance assigned to $Trp^{3}C(2)H$ and the next largest decrease observed was in the resonance assigned to $Tyr^{5}C(2,6)H$. The spectra of the aromatic region which we obtained from a freshly prepared sample are shown in Figure 1. Over a period of hours at 25C the difference signal gradually decreased. After eight hours it was barely discernable in the noise. The difference signal was restored to nearly its full value after the sample was dried under high vacuum and redissolved in solvent.

The nuclear Overhauser effects which we expected to observe were calculated from the two lowest energy conformations of LHRH (AA and CC) (1,2) using a method described by Noggle and Schirmer (13). For the purposes of the calculation we estimated τ_c from the molecular dimensions. The magnitude of the maximum NOE is very sensitive to the choice of τ_c so we cannot expect to derive distances from the magnitudes of the NOE we observe. However, we can expect to deduce conformational information from the rel-

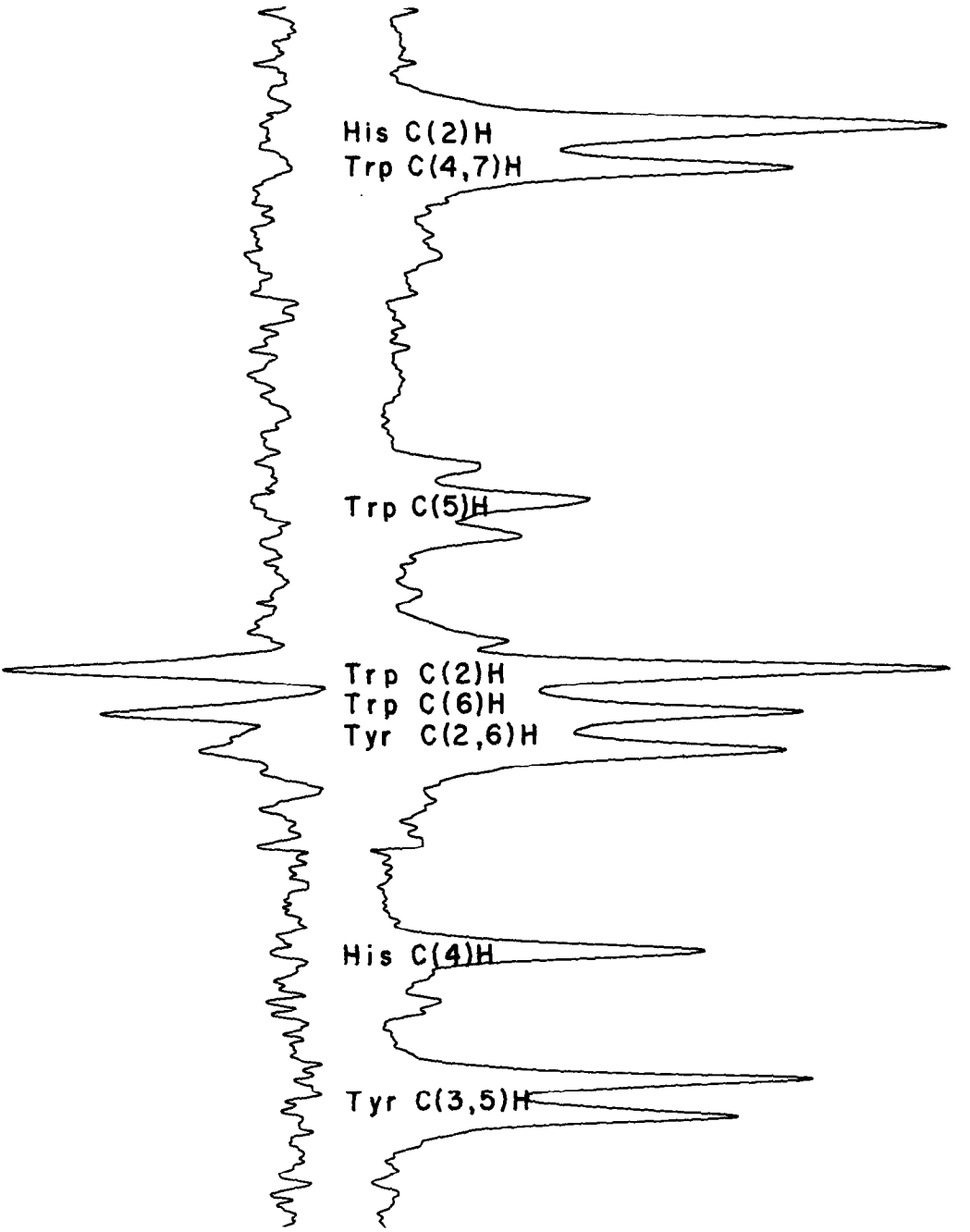

Figure 1. NOE obtained by saturating the resonances at 2.3ppm in a D_2O solution of LHRH. Upper trace, aromatic resonances between 7.6ppm and 6.8ppm with the saturating field offset from resonance; Lower trace, difference spectrum obtained by subtracting the upper trace from the spectrum obtained with the saturating field at 2.3ppm. Each spectrum was obtained from the sum of 50 traces, each taken in 2.5s over a frequency range of 500Hz. The results were transformed as described by Dadok and Sprecher (11) and smoothed with a 0.3Hz numerical filter.

ative magnitudes of two or more effects in the same molecule. The results of our calculations on conformers AA and CC are presented in Table I for the case where the resonances due to the $pGlu^{1}C\beta,\gamma$ and $Pro^{9}C\beta$ protons (2.3ppm) were saturated. For the purposes of the calculation τ_c was taken to be 1.5×10^{-9}s and relaxation by the solvent was taken to be at a rate of $0.02s^{-1}$. All protons in the molecule were included in the calculation of spin polarizations as we could find no rationale for excluding some of them. The calculated effects are primarily those due to the $pGlu^{1}C\beta,\gamma$ protons, as separate calculations in which only those protons were saturated gave substantially the same results for the aromatic protons.

We infer several things from a comparison of the experiment with the calculations. First, τ_c is approximately of the correct order of magnitude because we predict the correct sign of the NOE. Second, there are probably at least two conformers in solution because no energetically reasonable model predicts a negative NOE for both the $Trp^{3}C(2)H$ and the $Tyr^{5}C(2,6)H$ protons on saturation of the resonances near 2.3ppm. Third, the backbone conformations present probably resemble conformers AA and CC. We attribute the loss of the effect after a time to aggregation of the peptide. Such a slow aggregation was recently reported for neutral Pro-Leu-Gly-NH_2 in water solution (14). Such aggregation could easily lead to the introduction of additional relaxation which could reduce the magnitude of the NOE.

We find from a study of a molecular model of conformer AA that rotation of the Trp^{3} side chain through a small angle brings the structure into congruence with the observation of an NOE for the $Trp^{3}C(2)$ proton but none for the $Trp^{3}C(5,6)$ protons. A molecular model of conformer CC indicates that the magnitude of the

Table I. Calculated NOE for LHRH Upon Saturation of pGluCβ,γ and ProCβ Protons at 2.3ppm in D_2O or 2.0ppm in DMSO

CONFORMER CC			
DETECTED PROTON	SHIFT(ppm) [1] DMSO	D_2O	ENHANCEMENT(%) [2]
LEU CαH + ARG CαH	4.4	-	-13.5
TYR C(2,6)H	7.03	7.11	- 9.5
TYR C(3,5)H	6.64	6.81	- 8.0
ARG Cβ,γ H+ LEU Cβ,γH	1.5	1.6	- 3.3
SER CαH	4.35	-	- 3.2
SER CβH	3.60	3.71	- 1.6

CONFORMER AA			
DETECTED PROTON	SHIFT(ppm) [1] DMSO	D_2O	ENHANCEMENT(%) [2]
TRP C(4)H	7.60	7.57	-17.8
TRP C(2)H	7.15	7.15	- 6.5
TRP C(5)H	6.99	7.0	- 6.1

1. Shifts are those reported by Wessels et.al. (6) and referenced to internal TMS in DMSO and internal DSS in D_2O.
2. Enhancements are reported as defined by Noggle and Schirmer (13).

NOE expected for the $Tyr^5C(2,6)$ protons can be increased at the expense of the effect for the $Tyr^5C(3,5)$ protons by a small rotation of the Tyr^5 side chain. Thus, minor conformational changes in the side chains, which are at low energy in the calculations, should bring detailed conformational NOE calculations into congruence with the experiment. We have predicted several other feasible NOE measurements for this molecule and are in the process of doing the experiments. The details of the calculations and measurements will be presented elsewhere.

REFERENCES

1. Momany, F.A. (1976) J. Am. Chem. Soc. 98,2990-2996.
2. Momany, F.A. (1976) J. Am. Chem. Soc. 98,2996-3000.
3. Momany, F.A. (1978) J. Med. Chem. 21,63-68.
4. Deslauriers, R., Walter, R., and Smith, I.C.P. (1973) Biochem. Biophys. Res. Commun. 53,244-250.
5. Deslauriers, R., Komoroski, R.A., Levy, G.C., Seely, J.H., and Smith, I.C.P. (1976) Biochemistry 15,4672-4675.
6. Wessels, P.L., Feeney, J., Gregory, H., and Gormley, J.J. (1973) J. Chem. Soc. Perkins Trans.2. 12,1691-1698.
7. Howarth, O.W. (1978) J. Chem. Soc. Faraday Trans.2. 74,1031-1041.
8. Mabry, S., and Klotz, I.M. (1976) Biochemistry 15,234-242.
9. Marche, P., Montenay-Garesteir, T., Helene, C., and Fromageot, P. (1976) Biochemistry 15,5730-5737.
10. Marche, P., Montenay-Garesteir, T., Helene, C., and Fromageot, P. (1976) Biochemistry 15,5738-5743.
11. Dadok, J., and Sprecher, R.F. (1974) J. Magn. Res. 13,243-248.
12. Baldescheiler, J.D. (1964) J. Chem. Phys. 40,459-472.
13. Noggle, J.H., and Schirmer, R.E. (1971) The Nuclear Overhauser Effect, Academic Press, New York.
14. Walter, R., Deslauriers, R., and Smith, I.C.P. (1978) FEBS Lett. 95,357-360.