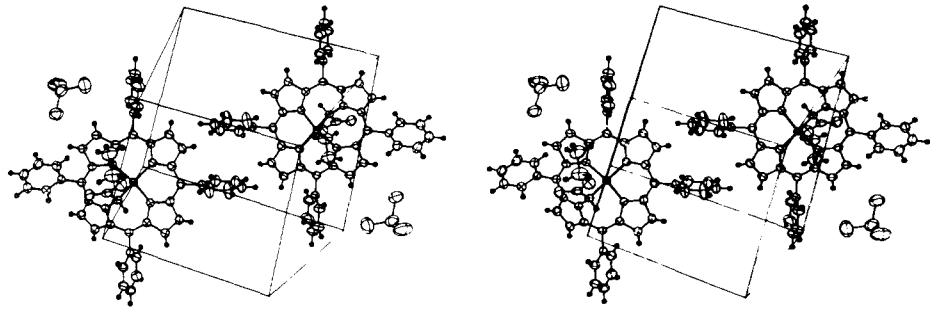

Figure 3. Molecular content of the unit cell (stereoscopic view). The thermal ellipsoids are drawn for 50% probability, except for those of the hydrogen atoms which are not drawn to scale.

contributes to increase cohesion between successive porphyrin complexed molecules.

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Supplementary Material Available. A listing of structure factor amplitudes (11 pp). Ordering information is given on any current masthead page.

References and Notes

(1) H. J. Callot, A. W. Johnson, and A. Sweeney, *J. Chem. Soc., Perkin Trans. 1*, 1424 (1973).
(2) P. Batten, A. Hamilton, A. W. Johnson, G. Shelton, and D. Ward, *J. Chem. Soc., Chem. Commun.*, 550 (1974).
(3) H. J. Callot, *Bull. Soc. Chim. Fr.*, 4387 (1972).
(4) H. J. Callot and T. Tschamber, *Bull. Soc. Chim. Fr.*, 3192 (1973).
(5) H. J. Callot and T. Tschamber, *Tetrahedron Lett.*, 3155, 3159 (1974).
(6) H. J. Callot, T. Tschamber, B. Chevrier, and R. Weiss, *Angew. Chem.*, **87**, 545 (1975).
(7) B. Chevrier, and R. Weiss, *J. Am. Chem. Soc.* **97**, 1416 (1975).
(8) A. W. Johnson, *Chem. Soc. Rev.*, 1 (1975).
(9) W. R. Busing, "Crystallographic Computing", F. R. Ahmed, Ed., Munksgaard, Copenhagen, 1970, p 319.
(10) D. T. Cromer and J. T. Waber, *Acta Crystallogr.*, **18**, 104 (1965).
(11) "International Tables for X-ray Crystallography", Vol. III, Kynoch Press, Birmingham, 1962, p 215.
(12) C. T. Prewitt, "A Fortran IV Full-Matrix Crystallographic Least-Squares Program", SFLS 5, 1966.
(13) See paragraph at end of paper regarding supplementary materials.
(14) C. K. Johnson, Program ORTEP, ORNL 3794, 1965.
(15) L. Pauling, "The Nature of the Chemical Bond", 3rd ed, Cornell University Press, Ithaca, N.Y., 1960.
(16) B. L. Barnett and C. Kruger, *J. Organomet. Chem.* **42**, 169 (1972).
(17) E. B. Fleischer, *J. Am. Chem. Soc.*, **85**, 146 (1963).
(18) T. A. Hamor, W. S. Caughey, and J. L. Hoard, *J. Am. Chem. Soc.*, **87**, 2305, (1965).
(19) D. L. Cullen and E. F. Meyer, Jr., *J. Am. Chem. Soc.*, **96**, 2095 (1974).
(20) V. Schomaker, J. Waser, R. E. Marsh, and G. Bergman, *Acta Crystallogr.*, **12**, 600 (1959).
(21) J. L. Hoard, *Science*, **174**, 1295 (1971).
(22) J. L. Hoard, *Ann. N.Y. Acad. Sci.*, **206**, 18 (1973).

Conformational Energy Analysis of the Molecule, Luteinizing Hormone-Releasing Hormone. 1. Native Decapeptide

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Abstract: Low-energy conformations of luteinizing hormone-releasing hormone (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) have been obtained using "empirical" energy calculations. The conformational space of the sections <Glu^1 to Gly^6 and Gly^6 to Gly^{10}-NH_2 was searched as separate entities, and the minimum energy conformations of these two sections were then used as starting conformations for refinement of the complete molecule. The minimum energy structure is consistent with experimental assay data derived from a series of amino acid substitution analogues.

Since the characterization of the luteinizing hormone-releasing hormone (LH-RH) molecule, <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, as the factor responsible for stimulating the secretion of the pituitary hormone(s) which regulate ovulation,[1-3] many synthetic analogues have been assayed in an attempt to illucidate the structure-activity relationships and to search for inhibitors of LH-RH.[4-9] A structure for LH-RH has been proposed,[10] based on model building and using experimental analogue data. Two carbon-13 NMR studies[11,12] have given some insight into selected aspects of the conformation of LH-RH, and a proton NMR study has added some understanding to the conformation.[13] However, to date, the conformation(s) remain unknown for LH-RH, either in solution or in the solid state. In order to gain some further understanding of the stereochemistry of LH-RH, empirical energy calculations[14-16] were carried out to search for the most stable conformation(s) of LH-RH.

LH-RH is not a small polypeptide from the standpoint of conformational studies (see Figure 1), and it is not feasible to attempt to examine *all* of its conformational space by generating all conformations and comparing their intramolecular energies. However, by using the observation that D-Ala substituted in place of glycine at the 6 position[6,17] causes a conformational stabilization which greatly enhances the production of luteinizing hormone, while L-Ala at the 6 position reduces the LH-RH potency to ~4% of the natural LH-RH, it is possible to reduce the conformational space of Gly^6 to a region defined by two possible sets of backbone dihedral angles around the Gly^6 residue. Further, a carbon-13 NMR study[11] indicated that the proline peptide bond was completely trans in LH-RH. Using these two

Table I. Starting Conformations for Each Amino Acid Residue in LH-RH

Amino acid residue	Conformations
<Glu	β_1, ±60°
His, Trp, Ser, Tyr, Leu, Arg	C_7^{eq}, β_1, β_2, 2_1, α_R
Gly^6	C_7^{ax}, α_L
Gly^{10}	C_7^{ax}, C_7^{eq}, β_1, α_R, α_L
Pro	I ($\psi = -15 \rightarrow -50°$), II ($\psi = 150 \rightarrow 170°$)

Conformation	Range of backbone dihedral angles[a]
C_7^{eq}	$\phi = -80 \pm 20°$; $\psi = +80 \pm 20°$
C_7^{ax}	$\phi = +80 \pm 20°$; $\psi = -80 \pm 20°$
β_1	$\phi = -165 \pm 15°$; $\psi = 155 \pm 25°$
2_1	$\phi = -145 \pm 5°$; $\psi = 75 \pm 5°$
β_2	$\phi = -70 \pm 10°$; $\psi = 130 \pm 10°$
α_R	$\phi = -70 \pm 10°$; $\psi = -60 \pm 10°$
α_L	$\phi = 70 \pm 10°$; $\psi = 60 \pm 10°$

[a] The uncertainty values in dihedral angles cover the range of minimum energy positions found in each given dipeptide conformation[15] for all the residues listed.

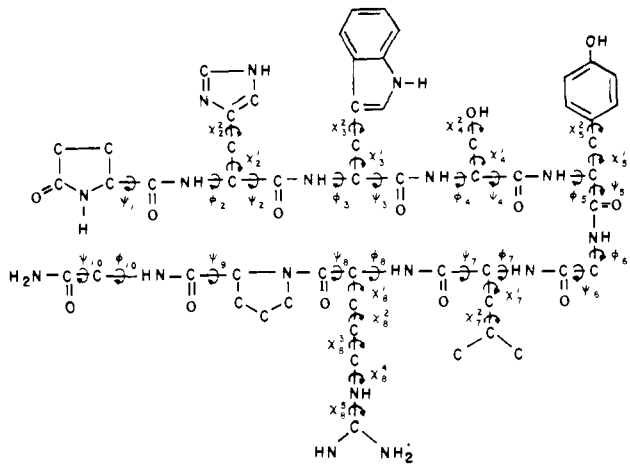

Figure 1. Primary sequence of luteinizing hormone-releasing hormone showing dihedral angles allowed to vary in the energy minimization. Some hydrogen atoms have been omitted for clarity.

pieces of experimental information, as well as the computationally derived low-energy "dipeptide" conformations of the residues involved,[15] it has been possible, by energy minimization techniques, to find three low-energy conformations of LH-RH, one of which appears to explain the observed analogue data.

Energy Calculations. The empirical energy parameters and functions used for this analysis have been documented elsewhere,[15,16] and only a brief description of these calculations will be given here. The conformational energy has been partitioned into various nonbonded, electrostatic, and hydrogen bonding interactions, plus torsional energy (arising from rotations around bonds). The attractive nonbonded energy terms interacting between atoms have been obtained from atomic polarizability data and the repulsive terms derived from crystal packing studies.[18] Electrostatic energy was obtained from the pairwise coulombic interactions of partial atomic charges (monopole approximation), which were determined from CNDO/2 molecular orbital calculations[19] on amino acids.[15] An "effective" dielectric constant of 2 is used throughout. The stabilization energy arising from the formation of hydrogen bonds was calculated using a 10–12 function,[18,20] and the parameters of this term were refined by crystal packing analysis.[18] Interactions between atoms separated by three bonds are treated differently than those separated by four or more bonds.[15,20] The bonds which require a torsional term include the peptide bonds, the hydroxyl C–O bond of Thr, the hydroxyl C–O bond of Ser, and the χ^5, χ^6, and χ^7 bonds of Arg (see Figure 1). The imidazole ring of His was taken to have the proton located on the N^δ position, and Arg was taken as uncharged. The <Glu geometry was that described previously for calculations carried out on thyrotropic releasing factor (TRF),[16] with the structure corrected to give the L isomer.[21] The proline geometry was that described previously.[16]

Choice of Starting Conformations. In order to determine a conformation which is of moderately low energy (i.e., has no serious atomic overlaps of very high energy for short-range interactions), it is possible to use the results of energy minimization calculations on dipeptides.[15] The dipeptide minima of low energy which were used here to generate starting conformations are given in Table I. The dihedral angles are given in IUPAC–IUB convention[22] throughout this work. Because of the very large number of starting conformations which are allowed from the dipeptide values given in Table I, some simplifying assumptions were necessary. The first simplification was to use molecular models[23] as tests for each starting conformer. If the conformer was such that residues separated by at least one residue showed atomic overlaps which could not be reduced by minor adjustments (i.e., ±20°) of backbone dihedral angles, then that conformer was discarded. Thus, only conformers which did not exhibit excluded volume effects (i.e., serious long-range repulsive interactions) were chosen to be studied by energy minimization. A more detailed discussion of this procedure will be given for the two individual sections of LH-RH (i.e., residues 1–6 and 6–10) which were studied separately. The low-energy conformers of the two sections were subsequently combined to give the total sequence.

The sectioning of the molecule LH-RH into two parts was carried out in order to reduce the magnitude of the minimization problem (i.e., fewer variables at one time). The first section includes the residues from $<Glu^1$ through Gly^6. The second section includes Gly^6 through the N-terminal side.

The minimization technique used was that of the conjugate gradient algorithm proposed by Powell[24] and modified by Zangwill.[25]

Low-Energy Conformations of $<Glu^1$-His^2-Trp^3-Ser^4-Tyr^5-Gly^6-NH_2. The section $<Glu^1$ to Gly^6 was studied separately from the remaining residues. The N-terminal group was treated as an amide (Gly^6-NH_2). Experimental activity data[6,17] indicated that the conformation of the backbone at Gly^6 was acceptable for substitution of D-Ala^6, but not L-Ala^6; thus, Gly^6 was held in one of the conformations given in Table I. By starting Gly^6 preferentially in the C_7^{ax} and α_L conformations, there remain 18 variable dihedral angles associated with this section. The variables are ψ_1, ω_1 of <Glu (ϕ_1 is fixed by the geometry of the <Glu ring[14]); ϕ_2, ψ_2, χ_2^1, χ_2^2 of His^2; ϕ_3, ψ_3, χ_3^1, χ_3^2 of Trp^3; ϕ_4, ψ_4, χ_4^1, χ_2^2 of Ser^4; and ϕ_5, ψ_5, χ_5^1, χ_5^2 of Tyr^5. All ω's except that of <Glu were kept at the trans ($\omega = 180°$) conformation and χ_5^3 of Tyr^5 was held in the extended ($\chi_5^3 = 180°$) configuration. Starting conformations were constructed with the models from combinations of the favored values shown in Table I. The number of possible backbone conformations of this section before excluding overlapping regions is 1250. Starting at the C-terminal end, it was found that the Tyr^5-Gly^6-NH_2 pair preferred only six of the ten possible combinations (i.e., 40% are disallowed). This result was found by

Table II. Results after Ten Cycles of Energy Minimization upon <Glu-His-Trp-Ser-Tyr-Gly-NH_2

	Residue conformation[a]						
	<Glu[1], ψ, deg	His[2]	Trp[3]	Ser[4]	Tyr[5]	Gly[6]	Energy kcal/mol
A	125	β_2	β_2	C_7^{eq}	C_7^{eq}	C_7^{ax}	−10.2
B	167	β_2	β_2	α_R	2_1	C_7^{ax}	−9.6
C	126	C_7^{eq}	α_R	C_7^{eq}	α_R	C_7^{ax}	−9.3
D	176	α_R	C_7^{eq}	β_2	β_1	C_7^{ax}	−9.2
E	125	β_2	β_2	β_2	β_2	C_7^{ax}	−8.5
F	93	α_R	β_2	C_7^{eq}	C_7^{eq}	C_7^{ax}	−8.5
G	125	β_2	β_2	C_7^{eq}	2_1	C_7^{ax}	−7.3
H	−176	α_R	α_R	C_7^{eq}	2_1	α_L	−6.2
I	169	α_R	α_R	α_R	α_R'[b]	α_L	−5.0
J	140	α_R	β_2	β_2	β_2	C_7^{ax}	−3.7
K	162	β_2	β_2	α_R	β_1	C_7^{ax}	−2.7

[a] See Table I for specification of the conformational regions in terms of backbone dihedral angles. [b] α_R' was not a starting conformation and resulted from energy minimization (α_R' is defined as $\phi = -140 \rightarrow -150°$; $\chi = -50 \rightarrow -60°$).

Table III. Results after Ten Cycles of Energy Minimization upon *N*-Ac-Gly-Leu-Arg-Pro-Gly-NH_2

	Residue conformation					
	Gly[6]	Leu[7]	Arg[8]	Pro[9], ψ, deg	Gly[10]-NH_2	Energy, kcal/mol
a	C_7^{ax}	α_R	2_1	−20	β_1	−40.5
b	C_7^{ax}	C_7^{eq}	2_1	−17	β_1	−38.1
c	C_7^{ax}	α_R	2_1	165	β_1	−38.0
d	α_R	α_R	2_1	−43	C_7^{ax}	−37.7
e	C_7^{ax}	C_7^{eq}	2_1	−49	C_7^{eq}	−37.6
f	C_7^{ax}	C_7^{eq}	2_1	−46	C_7^{ax}	−36.4
g	α_L	C_7^{eq}	2_1	−22	β_1	−36.4
h	α_L	α_R	2_1	−22	β_1	−36.3
i	C_7^{ax}	2_1	2_1	−45	C_7^{ax}	−36.0
j	C_7^{ax}	C_7^{eq}	2_1	165	β_1	−35.9
k	α_R	2_1	2_1	−18	α_R'[a]	−35.4
l	C_7^{ax}	2_1	β_2	−55	C_7^{ax}	−35.0
m	β_1	β_1	β_1	−50	C_7^{ax}	−30.1

[a] See Table II for definition of α_R' conformation.

computing the energies for all ten combinations and was found to be nearly independent of the Ser[4] conformation. The Trp[3] and Ser[4] conformations were even more sterically sensitive than the Tyr[5]-Gly[6]-NH_2 since variation in their backbone ϕ and ψ values could bring the ends of the molecule together causing many long-range steric overlaps. The large Trp[3] side chain directed the exclusion of certain conformations farther along the chain. The His[2] conformation was also limited to certain allowed combinations of ϕ and ψ, while the <Glu[1] ψ value was moderately free to rotate (in the regions $\psi = \pm 60$ and 180°) and this residue took up conformations which were most energetically preferred from long-range interactions. A total of ~200 conformations was found to have acceptable backbone conformations from model building studies. The side chains were allowed to overlap in these initial models. Upon constructing the 200 allowed conformers, the side chains of each particular residue were put into the most favored conformation found in dipeptide studies, for the particular backbone ϕ and ψ values of the given residue. Energy minimization was subsequently carried out, including all 20 variables, until the particular starting conformation either could not find a path to a lower energy form or until the energy did not change from one cycle to the next by more than several tenths of a kcal/mol. Of the 200 starting conformations, only those listed in Table II were found to be of energy less than 8 kcal/mol higher than the lowest energy conformer found. In each of the low-energy structures of Table II, several side-chain conformer combinations were further tested to verify that the most favorable side-chain positions had been found. In almost every case, it was found that the best side-chain conformation for the "dipeptide" was also the best for the polypeptide. The conformations and energies resulting from the above procedure for the first six residues, after at least ten cycles of energy minimization (i.e., through ~500 energy function calculations), are given in Table II. No obvious trends stand out insofar as a particular residue conformation is concerned. Thus, all of the conformations of Table II are combined with the conformations obtained in the next section, in order to obtain the lowest energy conformer for the complete LH-RH molecule.

Low-Energy Conformations of *N*-Ac-Gly[6]-Leu[7]-Arg[8]-Pro[9]-Gly[10]-NH_2. The section of LH-RH starting at Gly[6] was also studied independently of the first five residues. As in the previous section, it was possible to fix the ϕ and ψ values of Gly[6] at one of the two conformations of Table I, and by doing this, it was possible to overlap this residue with the results for Gly[6] of the previous section. The 14 variable dihedral angles to be examined in this section are ϕ_6, ψ_6 of Gly[6]; ϕ_7, ψ_7, χ_7^1, χ_7^2 of Leu[7]; ϕ_8, ψ_8, ω_8, χ_8^1, χ_8^4 of Arg[8]; ψ_9 of Pro[9]; and ϕ_{10}, ψ_{10} of Gly[10]. The dihedral angles ω_6, ω_7, ω_9, and ω_{10} are all taken to be in the trans (ω = 180°) conformation, while χ_7^3 and χ_7^4 of Leu[7] and χ_8^2, χ_8^3, χ_8^5, χ_8^6, and χ_8^7 of Arg[8] are kept in the extended (χ = 180°) conformation. Similarly to the previous section, possible starting conformations of this section were constructed from combinations of the favorable values of each backbone dihedral angle, and the side-chain conformations were chosen to be those of low energy for a given dipeptide backbone values.[15] Models of the various starting conformations were next examined for long-range atomic overlap, and those models found to be acceptable were generated and put through a series of energy minimization cycles in which all the above dihedral angles were allowed to vary. The results of this study are given in Table III. Only the 13 lowest energy conformations (obtained after at least ten cycles of energy minimization) are given. Many other conformations were eliminated by model building and others by energy considerations.

Several observations which can be made from Table III are as follows. First, all lowest energy conformations have Arg[8] in the 2_1 backbone arrangement. This result appeared even when the starting values of ϕ and ψ of Arg[8] were *not* those of the 2_1 conformers. In fact, in only a few cases (all more than 5 kcal/mol above the lowest energy conformer) did Arg[8] remain in a conformation other than 2_1 (see last two entries of Table III). Second, Leu[7] was found to prefer the α_R conformation (as in a) over that of C_7^{eq} (as in b) by ~2 kcal/mol. Third, Pro[9] was found to prefer form I over form II by several kcal/mol in this short section of the molecule. Tests of χ_7^1 of Leu[7] and χ_8^1 of Arg[8] were made to ascertain that the best "dipeptide" side-chain positions[15] were in fact also the best for the oligomer studied here.

Low-Energy Conformation of LH-RH. The results from the two previous sections were used to construct models of the complete LH-RH molecule. All possible combinations of A–K of Table II and a–m of Table III were constructed. It was found that many combinations could be eliminated because of extensive atom overlaps which could not be reduced by small changes (i.e., ±20 in ϕ and ψ) in any backbone dihedral angles. In those cases where side-chain atoms

Table IV. Results of Energy Minimization on <Glu-His-Trp-Ser-Try-Gly-Leu-Arg-Pro-Gly-NH_2

	Residue conformation										
No. of starts	<Glu[1], ψ, deg	His[2]	Trp[3]	Ser[4]	Tyr[5]	Gly[6]	Leu[7]	Arg[8]	Pro[9], ψ, deg	Gly[10]	Energy, kcal/mol
AA 9	173	C_7^{eq}	β_2	C_7^{eq}	C_7^{eq}	C_7^{ax}	2_1	2_1	−17	β_1	−50.0
BB 5	170	C_7^{eq}	β_2	C_7^{eq}	C_7^{eq}	C_7^{ax}	2_1	2_1	173	β_1	−49.0
CC 7	137	α_R	β_2	C_7^{eq}	C_7^{eq}	C_7^{ax}	2_1	2_1	−27	β_1	−47.2
DD 2	156	α_R	C_7^{eq}	2_1	β_1	C_7^{ax}	α_R	2_1	−17	β_1	−43.7
EE 10	−179	β_2	β_1	α_R	C_7^{eq}	β_2'[b]	2_1	2_1	−28	β_1	−40.8
FF 3	172	α_R	β_2	C_7^{eq}	C_7^{eq}	C_7^{ax}	2_1	2_1	177	α_R'[a]	−39.3
GG 2	172	α_R	α_R	C_7^{eq}	C_7^{eq}	$2_1'$[c]	C_7^{eq}	2_1	−23	β_1	−37.5
HH 1	172	α_R	β_2	C_7^{eq}	α_R	C_7^{ax}	α_R	2_1	−54	C_7^{eq}	−36.5
II 1	126	β_2	β_2	C_7^{eq}	C_7^{eq}	α_L	2_1	2_1	−23	β_1	−35.9

[a] See Table II for the definition of the α_R' conformation. [b] The β_2' conformation has dihedral angles of $\phi = 70 \pm 10°$; $\psi = -130 \pm 10°$. [c] The $2_1'$ conformation has dihedral angles of $\phi = 140 \rightarrow 150°$; $\psi = -70 \rightarrow -80°$.

were sterically interacting with atoms of the second section, changes in side-chain dihedral angles were examined in order to reduce these overlaps. The variables for energy minimization are the same as those used for both previous sections of the molecule, plus the added variables, ω_6 of Gly[6], ω_7, χ_7^3, χ_7^4 of Leu[7], and χ_8^2, χ_8^3, χ_8^5 of Arg[8]. A total of 39 dihedral angles were allowed to vary in the minimization cycles carried out on LH-RH. The number of different starting conformations in which all variables were allowed to be varied, and on which at least three cycles of energy minimization were carried out, was 143. Of these 143 starting conformations, the lowest energy conformers were further minimized until the total energy change was less than 0.1 kcal/mol per cycle.

The results are given in Table IV and the number of starts indicates the number of different side-chain conformers tried for the given backbone conformation. In many cases, the starting conformation was considerably perturbed upon energy minimization. An example of the magnitudes of change in dihedral angles that occurred for each residue upon ten cycles of energy minimization is shown in Figure 2. In the case shown (EE of Table IV), the backbone dihedral angles of the Leu[7] residue were started near the α_R conformation and, after minimization, resulted in a conformation in the 2_1 region. This conformational change of ~90° in ψ involved going through an energy pass and over a barrier of several kilocalories. At each step in this change, however, the total conformational energy was decreasing. Other changes in dihedral angle for this case are of the order of 10 → 30° in ϕ or ψ, and changes of this magnitude were observed in many of the conformations given in Table IV.

The lowest energy conformations found for LH-RH are AA, BB, and CC. An examination of the combination of the two sections of LH-RH which were studied independently shows that upon combining two low-energy sections some changes in residue conformation take place. For example, the His[2] residue in the β_2 conformation of conformer A in Table II has been converted to a C_7^{eq} conformation in LH-RH, (i.e., see AA of Table IV). Also, conformation b of Table III is close to that found in the lowest energy conformation of LH-RH, except that Leu[7] (C_7^{eq}) has moved into the 2_1 region for this residue. These changes are not large (see Figure 2) and do not entail significant changes in energy. However, it is of interest to note that the complete molecule of LH-RH was required in order for these conformations to become of lower energy than the starting (i.e., sectioned) conformations.

Structure CC of Table IV is similar in most respects to structures AA and BB, being made up of conformer F of

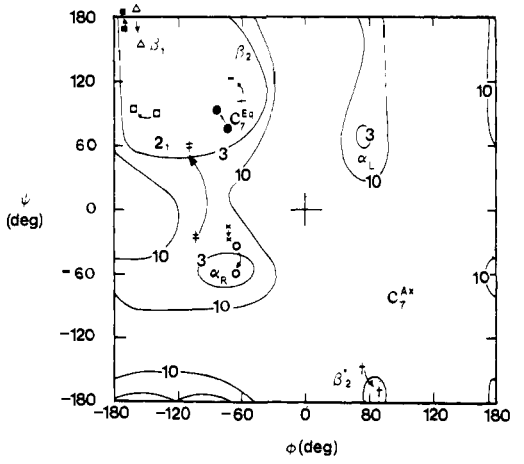

Figure 2. A $\phi(N\text{-}C^\alpha)$, $\psi(C^\alpha\text{-}C')$ conformational energy contour diagram for the molecule *N'*-methyl-L-alanylamide. The arrows indicate the direction that each set of backbone ϕ and ψ values took, upon energy minimization, to obtain conformer EE: His[2] (+), Trp[3] (△), Ser[4] (○), Tyr[5] (●), Gly[6] (†), Leu[7] (‡), Pro[9] (×), and Gly[10] (■). The energy is in kcal/mol.

Table II and b, modified as described above for AA, of Table III. Other combinations of the two sections are seen in the higher energy structures of Table IV, but because of the large gap (~7 kcal/mol) in energy from structure AA to DD, we will limit ourselves to a discussion of conformers AA through CC.

The partitioned energy components of conformers AA and CC show no striking differences. For example, the electrostatic energy is −1.5 kcal/mol in AA and −1.2 kcal/mol in CC, while the nonbonded (repulsive + attractive) plus hydrogen bonded energies are −48.8 kcal/mol in AA and −47.5 kcal/mol in CC. The torsional energy terms (+0.3 kcal/mol in AA and +1.5 kcal/mol in CC) differ in large measure from the increased nonplanarity of the Arg[8]-Pro[9] peptide bond in conformer CC, as compared to this bond in AA. The low values for the torsional energy indicate that no serious strain has been put on those bonds which have a torsional potential contribution.

The dihedral angles for conformers AA and CC of Table IV are given in Table V, and structure AA is shown in Figure 3. Structure CC is shown in paper 2[26] of this series. The only major difference between structures AA and BB is in the ψ value of Pro[9]. This change in ψ(Pro) moves the tail (Gly^{10}-NH_2) of the molecule as shown in Figure 4, and these positions which are energetically closer in the complete LH-RH molecule than they were in the sectional sequence will be discussed in paper 2.[26]

Table V. Low-Energy Conformations of LH-RH[a]

	Residue	Dihedral angles, deg									
		ϕ	ψ	ω	χ_1	χ_2	χ_3	χ_4	χ_5	χ_6	χ_7
					Conformation AA (ψ – Pro^9 = −17.5°)						
1	Pyroglutamate		173.0	−179.0							
2	Histidine	−87.6	87.1	180.0	−160.0	−62.4					
3	Tryptophan	−79.7	166.6	180.0	−60.2	−70.9					
4	Serine	−74.2	94.6	180.0	66.9	50.6					
5	Tyrosine	−79.7	88.1	180.0	−54.8	122.0	180.0				
6	Glycine	80.8	−76.2	177.3							
7	Leucine	−149.6	49.0	−174.5	−158.3	86.0	−171.7	−174.6			
8	Arginine	−151.9	87.6	177.5	−164.6	165.9	170.0	−78.4	178.7	180.0	180.0
9	Proline-down-B	−75.0	−17.5	180.0							
10	Glycine	−159.5	152.6	180.0							
11	Carboxyl-NH_2			180.0							
					Conformation CC (ψ – Pro^9 = −28.5)						
1	Pyroglutamate		137.0	179.3							
2	Histidine	−68.0	−48.2	180.0	−158.4	−56.8					
3	Tryptophan	−123.9	163.7	180.0	−63.4	−75.4					
4	Serine	−76.9	95.3	180.0	67.2	50.2					
5	Tyrosine	−82.2	96.7	180.0	−56.9	−52.7	180.0				
6	Glycine	79.8	−91.4	170.5							
7	Leucine	−138.7	58.4	179.5	−161.9	86.2	−171.6	−174.6			
8	Arginine	−152.2	85.6	167.1	−166.6	−175.3	177.4	75.8	−176.0	180.0	180.0
9	Proline-down-B	−75.0	−28.5	180.0							
10	Glycine	−144.9	142.5	180.0							
11	Carboxyl-NH_2			180.0							

[a] E_0 = −50.0 kcal/mol for conformation AA and E_0 = −47.2 kcal/mol for conformation CC.

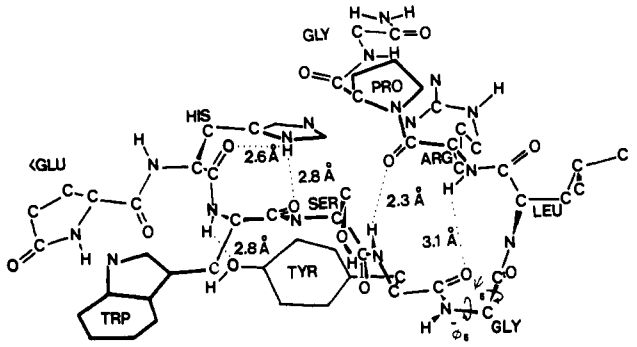

Figure 3. View of conformer AA. For clarity, some of the hydrogen atoms have been omitted. The dotted lines indicate H···O hydrogen bond directions.

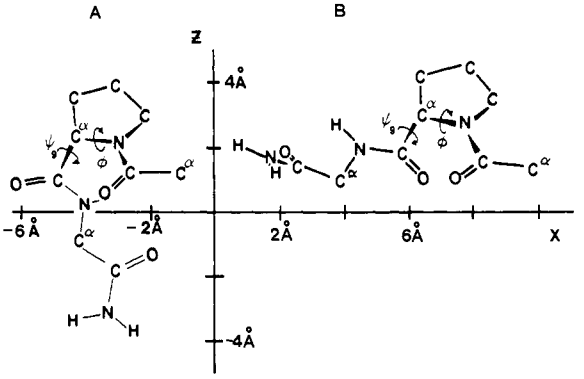

Figure 4. View of the two conformations around Pro^9. Conformer A is a "puckered" form, $\psi_9 \approx -20°$; B is an "extended" form, $\psi_9 \approx 165°$.

Conformation CC of Table IV differs from AA and BB primarily in the ψ value of His^2. The effect of this change is to reverse the His^2 ring and <Glu directions (see Figure 3),

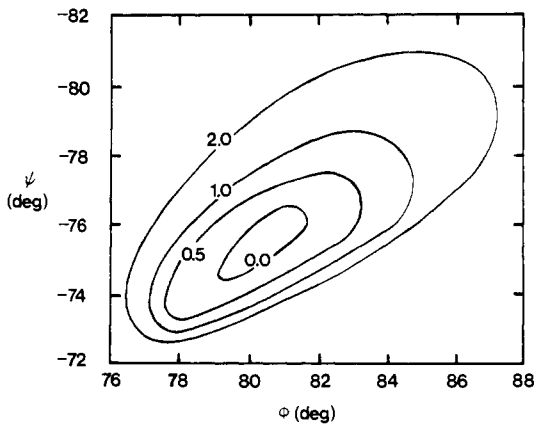

Figure 5. Energy contour diagram for change of energy of conformer AA, as ϕ_6 and ψ_6 of Gly^6 are varied. All other dihedral angles of conformer AA were held fixed. The energies are in kcal/mol, scaled to zero at the minimum of ϕ_6 = 80.8°; ψ_6 = −76.2°.

with the His^2 ring having nearly planar ring-ring stacking with the Trp^3 ring, but at a distance of C$^\gamma$(His)···C$^\gamma$(Trp) of ~4.5 Å and the <Glu^1 residue now being somewhat buried in nearly the same position as is the His^2 ring of structure AA, as shown in Figure 3. This conformation will be discussed further in the context of the structure-activity mechanism, in paper 2 of this series.[26]

Since structures AA–CC seem to be "hinged" at Gly^6, it was important to examine the flexibility of these conformers at this residue. As might be expected, the ease with which rotation can occur around the ϕ and ψ bonds of Gly^6 will play a major role in its overall conformational population in solution. In Figure 5 the total energy is shown as a function of the backbone dihedral angles, ϕ and ψ of Gly^6. From Figure 5 it can be seen that by opening up both angles

simultaneously one can move 3–5° along ϕ and ψ without losing more than 2 kcal/mol of energy. However, it is obviously not energetically favorable to open the structure up completely in this manner (i.e., this path for unfolding must go over a barrier of several kcal/mol). It does show, however, that if one substituted a bulky L isomer at this particular residue, steric effects would be sufficient to change the conformation around Gly[6].

The flexibility of various side-chain positions has also been examined. In particular, the position of the His[2] ring in conformer AA was examined for possible hydrogen bonding at the $N^{\delta}H$ or N^{ϵ} positions. It was found that no strong interactions at these sites were taking place and that by rotating χ_2^2 by ~180°, an equivalent energy conformer was obtained. Examination of the Trp ring conformation was also carried out for both AA and CC conformers, varying both χ_3^1 and χ_3^2. Equivalent energies for two conformers were found by minimization, with dihedral angles of $\chi_3^1 = -49°$, $\chi_3^2 = 107°$ and $\chi_3^1 = -60°$, $\chi_3^2 = -71°$. The first conformer, called "ring up", will be discussed further in paper 2, as a comparison to some analogue model compounds. Variation of the Ser[4], side-chain conformation gave the $\chi_4^1 = +66°$ and $\chi_4^2 = +50°$ as being best, due to a favorable hydrogen bond to the Ser[4] carbonyl oxygen. Changing χ_4^1 to −60° and χ_4^2 to 180° raises the energy by a few tenths of a kcal/mol, but this conformer would be allowed in solution. The side-chain dihedral angles of Leu[7] and Arg[8] which were not included in the energy minimization were also examined. The dihedral angle χ_8^4 of Arg was tested for +80° and found to be several tenths of a kcal/mol less favorable than the −80° conformer. The side chain of Leu was not found to be favorable in any other position.

Clearly, the side chains of LH-RH are relatively flexible and can take up different positions with little change in energy. Further, the small differences in energy between conformers AA, BB, and CC would indicate that some population of all three would exist in solution. However, the calculations presented here would indicate that the basic structural integrity of the backbone of the molecule will be retained as the largest population of the conformers in solution, and the structure should not be designated as a series of random conformations.

Conclusions

The low-energy conformers of LH-RH determined here (AA–CC) are consistent with the available experimental data. The results of a proton NMR study[13] gave J_{NH-CH} coupling constants in substantial agreement with the values of ϕ presented in Table V. That is, J_{NH-CH} values of 6.2–7.4 Hz give, from the Bystrov[27] relationship, values of ϕ in the range $-160 \pm 10°$ and $-80 \pm 10°$ (positive ϕ values are not included here although the Bystrov[27] relationship is degenerate in these angles). These values may be compared to the ϕ values of Table V. The Gly[6] ϕ value of ~150° is somewhat more difficult to analyze by the observed J_{NH-CH} values. However, it would appear that they are not incompatible with the observed J_{NH-CH} values of ~5.5 Hz.[13] The proton NMR data also indicated from temperature studies[13] that none of the NH protons of LH-RH were buried or involved in *strong* intramolecular hydrogen bonds. Figure 3 gives the closest hydrogen bond lengths for H···O interactions, and no short (i.e., ~1.7–2.0 Å) hydrogen bonds are found. Further, in conformer AA (Figure 3), all NH protons are accessible to water. This result is also in agreement with the experimental result.[13]

The $C^{\alpha}H$–$C^{\beta}H$ coupling constant for Ser[4] was also determined from the ^1H NMR study[13] and is in agreement with our favored value of $\chi_4^1 = +66°$ (i.e., rotamer III in ref 13). Further, the aromatic ring protons showed very little ring current effects,[13] indicating no appreciable stacking of the aromatic rings. This result is also in agreement with the three low-energy conformers obtained here. In fact, the rings of Trp and Tyr are nearly perpendicular to one another, and in conformer CC, in which the His ring is closest to the Trp ring, the distance between rings is still too large for significant ring current effects to be observed.

The study of the ^{13}C resonances[11,12] also indicated that the proline peptide bond was trans and further that *all* peptide bonds in the LH-RH molecule were trans.[12] The choice of all trans peptides in the calculations presented here is thus in agreement with the experimental data. Finally, the fact that no electrostatic intramolecular interactions were observed upon charging the imidazole ring at low pH[13] would favor conformation CC, where the His ring is pointing out into solution, away from the rest of the molecule.

It should be pointed out here that the use of uncharged His and Arg residues in these calculations is closely equivalent to using the charged residues when a shielding function is included to take account of the solvation effect. Since energies between different conformations of the same molecule are always compared, the effect of the solvent on the stabilizing energy is minimized. This is not to say that the solvent effect is not important to the total stabilization energy, but only that its effect on the conformation should be small in the case studied here.

It is worth noting that previous models of LH-RH have been proposed[28] with β bends starting at Ser in the ith position, Tyr in the $i + 1$, Gly in the $i + 2$, and Leu in the $i + 3$. In the low-energy structures reported here, Gly is in the $i + 1$ position, and Leu in the $i + 2$ position, with a modified type II bend being formed.

An evaluation of various analogues of LH-RH with respect to the conformers found here will be presented in paper 2,[26] and evidence will be presented which strongly implicates conformer CC as the biologically active conformer of LH-RH.

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References and Notes

(1) H. Matsuo, Y. Baba, R. M. G. Nair, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **43**, 1334 (1971).
(2) M. Monahan, J. Rivier, R. Burgus, M. Amoss, R. Blackwell, W. Vale, and R. Guillemin, *C. R. Hebd. Seances Acad. Sci., Ser. D*, **273**, 508 (1971).
(3) M. Amoss, R. Blackwell, and R. J. Guillemin, *Clin. Endocrinol. Metab.*, **34**, 434 (1972).
(4) J. Humphries, G. Fisher, Y. P. Wan, K. Folkers, and C. Y. Bowers, *J. Med. Chem.*, **17**, 569 (1974).
(5) M. W. Monahan, J. Rivier, W. Vale, R. Guilleman, and R. Burgus, *Biochem. Biophys. Res. Commun.*, **47**, 551 (1972).
(6) M. W. Monahan, W. Vale, J. Rivier, G. Grant, and R. Guillemin, Abstracts, 55th Meeting of the Endocrine Society, Chicago, Ill., 1973, p 145.
(7) Y. Okada, K. Kitamura, Y. Baba, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **53**, 1180 (1973).
(8) M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa, I. Yamazaki, R. Nakayama, W. F. White, and R. H. Rippel, *Biochem. Biophys. Res. Commun.*, **49**, 698 (1972).
(9) N. Yanaihara, K. Tsuji, C. Yanaihara, T. Hashimoto, T. Kaneko, H. Oka, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **51**, 165 (1973).
(10) J. K. Chang, R. H. Williams, A. J. Humphries, N. G. Johansson, K. Folkers, and C. Y. Bowers, *Biochem. Biophys. Res. Commun.*, **47**, 727 (1972).
(11) R. Deslauriers, R. W. Walter, and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, **53**, 244 (1973).
(12) R. Deslauriers, G. C. Levy, W. H. McGregor, D. Sarantakis, and I. C. P. Smith, *Biochemistry*, **14**, 4335 (1975).
(13) P. L. Wessels, J. Feeney, H. Gregory, and J. L. Gormley, *J. Chem. Soc., Perkin Trans. 2*, 1691 (1973).
(14) H. A. Scheraga, *Chem. Rev.*, 71, 195 (1971).
(15) P. N. Lewis, F. A. Momany, and H. A. Scheraga, *Isr. J. Chem.*, 11, 121 (1973).

(16) A. W. Burgess, F. A. Momany, and H. A. Scheraga, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 1456 (1973).
(17) M. Fujino, I. Yamazaki, S. Kobayashi, T. Fukuda, S. Shinagawa, R. Nakayama, W. F. White, and R. H. Rippel, *Biochem. Biophys. Res. Commun.*, **57**, 1248 (1974).
(18) F. A. Momany, L. M. Carruthers, R. F. McGuire, and H. A. Scheraga, *J. Phys. Chem.*, **78**, 1595 (1974).
(19) J. Yan, F. A. Momany, R. Hoffmann, and H. A. Scheraga, *J. Phys. Chem.*, **74**, 420 (1970).
(20) F. A. Momany, R. F. McGuire, A. W. Burgess, and H. A. Scheraga, *J. Phys. Chem.*, **79**, 2361 (1975).
(21) A. W. Burgess, F. A. Momany, and H. A. Scheraga, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 4640 (1974).
(22) IUPAC–IUB Commission on Biochemical Nomenclature, *J. Mol. Biol.*, **52**, 1 (1970).
(23) Nicholson, Molecular Models, LABQUIP, Caversham, Reading, RG48XE, England.
(24) M. J. D. Powell, *Comput. J.*, **7**, 155 (1964).
(25) W. I. Zangwill, *Comput. J.*, **10**, 293 (1967).
(26) F. A. Momany, *J. Am. Chem. Soc.*, following paper in this issue.
(27) V. F. Bystrov, S. L. Portnova, V. I. Tsetlin, V. T. Ivanov, and Yu. A. Ovchinnikov, *Tetrahedron*, **25**, 493 (1969).
(28) M. W. Monahan, M. S. Amoss, H. A. Anderson, and W. Vale, *Biochemistry*, **12**, 4616 (1973).

Conformational Energy Analysis of the Molecule, Luteinizing Hormone-Releasing Hormone. 2. Tetrapeptide and Decapeptide Analogues

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Abstract: Low-energy conformations of peptide analogues of luteinizing hormone-releasing hormone have been obtained using "empirical" energy calculations. The minimum energy conformations of the tetrapeptides, <Glu-Tyr-Arg-Trp-NH_2 (I) and <Glu-Trp-Arg-Tyr-NH_2 (II), are found and correlations with the native decapeptide noted. Several analogues of the native decapeptide are also calculated, using the native low-energy conformers as starting models. A discussion of the activity of luteinizing hormone-releasing hormone and various analogues is presented and compared to the structure of this molecule found from energy minimization techniques.

In the previous paper in this series[1] (paper 1), several minimum energy conformers of luteinizing hormone-releasing hormone (LH-RH) were found. Correlation of the calculated conformers with experimental analogue data was noted for the region of the molecule around the Gly^6 position. However, it was not possible to clearly distinguish between the two distinctly different low-energy structures (i.e., AA and BB vs. CC of Tables IV and V, paper 1) from analogue data. The conformational energy difference of 2.8 kcal/mol between AA and CC is not sufficient to exclude the CC conformer from consideration. Indeed, the CC conformer exposes the His^2 ring to solvent (or receptor surface) while putting the nonpolar portion of the <Glu ring into a shielded pocket in the structure. The added intramolecular energy associated with these changes may well be overcome by solvation effects or by intermolecular binding conditions at the receptor surface.

In this paper, evidence from tetrapeptide studies is presented that leads to the conclusion that structure CC is most probably the active conformer. Further calculations on various analogues of LH-RH are also presented and their influence on the conformations of structures AA and CC is examined.

Tetrapeptides. In vivo activity tests[2,3] have shown that <Glu-Tyr-Arg-Trp-NH_2 (I) has luteinizing hormone-releasing hormone (LH-RH) activity of ~1 part in 8000 of that exhibited by the natural LH-RH (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2). Of the other five permuted sequence tetrapeptides containing the terminal <Glu and the residues Arg, Tyr, and Trp, only molecule I showed any activity. It was not clear why molecule I should show some LH-RH activity, while the permutation of the two ring-bearing residues, as in the molecule <Glu-Trp-Arg-Tyr-NH_2 (II), lacked activity. Conformational energy calculations, as described in paper 1 of this series[1] were carried out to examine the conformational change that this difference in sequence might make and thus help identify the factors responsible for the overall mechanism of action of the series of LH-RH analogues and of the naturally occurring LH-RH molecule.

Since molecules I and II are small, relative to the natural LH-RH, and have only a few variable dihedral angles, it is possible to cover most of their conformational space by generating many starting conformations and refining each through a series of intramolecular energy minimization steps. Sixteen bonds were chosen, about which rotation can occur. The variable dihedral angles are denoted: ψ_1, ω_1 of <Glu; ϕ_2, ψ_2, χ_2^1, χ_2^2 of Tyr (or Trp); ϕ_3, ψ_3, χ_3^1, χ_3^2, χ_3^3, χ_3^4 of Arg; and ϕ_4, ψ_4, χ_4^1, χ_4^2 of Trp (or Tyr). The dihedral angle ϕ_1 of <Glu is fixed by the geometry of the pyroglutamate ring, and the ω angles of all the other residues were held fixed in the trans (ω = 180°) conformation. Possible starting conformations were generated from combinations of low-energy dipeptide conformations[4] and models constructed to examine long-range overlap. The conformations of molecules I and II, resulting from complete energy minimization of the 20 lowest energy conformations, which resulted from an initial set of ~80 starting conformations for each tetramer, are given in Table I. IUPAC–IUB[5] conventions are used to define the conformations. The minimization procedure and the amino acid geometry are described in paper 1. The arginine side chain was taken to be uncharged, and the position of the nitrogen lone pair varied by rotation of 180° about χ_3^5 and χ_3^6. The lowest energy conformations found for molecules I and II are shown in Figures 1 and 2, respectively. The values of the dihedral angles and the relative energy (ΔE) for the six lowest energy conformations of both molecules are given in Table I.

Results of Tetrapeptides. The low-energy structure of molecule I [<Glu-Tyr-Arg-Trp-NH_2] (A of Table I) is