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

F. A. Momany

Contribution from the Department of Chemistry, Memphis State University, Memphis, Tennessee 38152. *Received August 15, 1975*

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 ($\omega = 180°$) 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

Table I. Low-Energy Conformations of <Glu-Tyr-Arg-Trp-NH_2

Conformation	Dihedral angles, deg															ΔE,[a] kcal/mol
	ψ_1	ω_1	ϕ_2	ψ_2	χ_2^1	χ_2^2	ϕ_3	ψ_3	χ_3^1	χ_3^2	χ_3^3	χ_3^4	ϕ_4	ψ_4	χ_4^1	χ_4^2	
A	171	177	−85	83	−175	68	−77	−28	−69	173	−169	78	−152	152	39	75	0.0
B	176	179	−84	84	−175	71	−88	70	−67	−176	−167	78	−145	71	−56	99	1.2
C	173	177	−85	82	180	57	−80	95	−70	177	−166	78	−152	143	172	90	2.0
D	179	176	−68	154	180	82	−79	91	−171	−142	163	−78	−75	−48	−54	106	2.3
E	176	179	−84	84	−178	64	−88	73	−67	−177	−167	78	−156	144	−155	89	2.6
F	175	173	−75	142	−178	86	−139	−58	−80	177	−172	79	−97	142	59	85	3.0
Low-energy conformations of <Glu-Trp-Arg-Tyr-NH_2																	
A′	178	−178	−86	82	−166	76	−89	69	−66	−170	−166	78	−148	44	−59	100	0.0
B′	170	176	−86	84	180	68	−75	109	−72	172	−169	78	−151	149	−176	81	1.1
C′	177	−179	−89	81	−174	72	−84	92	−68	−176	−166	78	−152	148	−170	85	1.7
D′	169	−178	−87	88	−171	77	−78	−35	−70	169	−172	79	−155	160	50	81	1.7
E′	170	−179	−81	158	−164	75	−81	89	−172	−137	164	−80	−79	−36	−57	110	3.1

[a] E_0 for molecule I was −32.22 kcal/mol, E_0 for molecule II was −33.16 kcal/mol, $\Delta E = E_{conf} - E_0$.

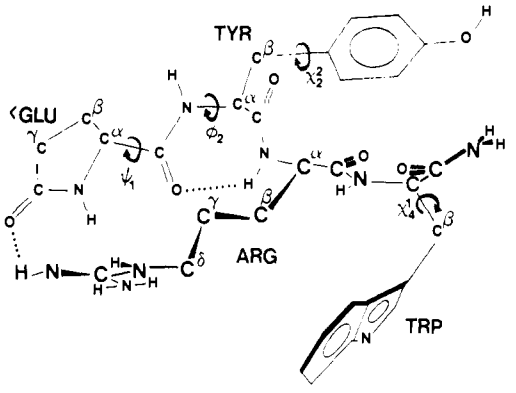

Figure 1. Lowest energy conformation of <Glu-Tyr-Arg-Trp-NH_2 (see A of Table I). The dotted line indicates the formation of a hydrogen bond.

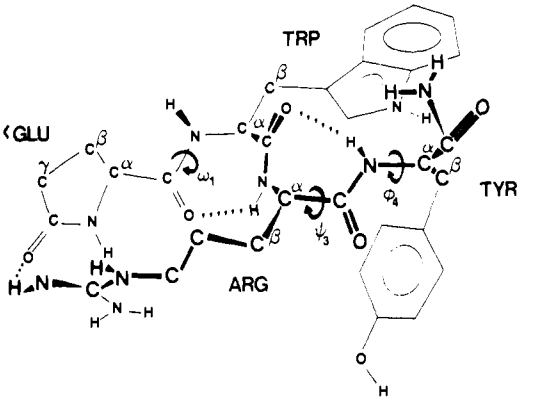

Figure 2. Lowest energy conformation of <Glu-Trp-Arg-Tyr-NH_2 (see A′ of Table I). The dotted line indicates the formation of a hydrogen bond.

shown in Figure 1. The carbonyl oxygen of the <Glu ring interacts by a hydrogen-bonding contribution to the arginine side chain, and this interaction tends to reduce the flexibility of the center section of the back-bone chain. Conformation A can be described as consisting of an equatorial seven-membered ring at Tyr, right-handed α helix at Arg, and extended at Trp. Conformation B, which is 1.2 kcal/mol less stable than A, has nearly the same <Glu to Arg side-chain interaction as in structure A. However, the Tyr and Trp rings are in closer contact (nearly plane to plane stacking), and the terminal $-NH_2$ now sticks up, similarly to that shown in Figure 2 for molecule II. Structure C differs from B only in the extension of the Trp ring (χ_4^1 = 174°) and the change in the terminal $-NH_2$ orientation (ψ_4 = 143°). Structure D has changed the <Glu to Arg relation such that the Arg side chain now points away from the <Glu ring, and the Trp side chain now is close to the <Glu ring.

The low-energy conformation of molecule II [<Glu-Trp-Arg-Tyr-NH_2] (A′ of Table I) is shown in Figure 2. The orientation of the <Glu ring to the Arg side chain is similar to that found for the low-energy conformation of molecule I. However, the conformation around the Tyr residue is close to that found for conformation B of molecule I. It can be seen from Table I that the conformation of molecule II, which most closely compares to conformation A of molecule I, is D′, which is 1.7 kcal/mol less stable than A′.

The most notable results of the calculations on these tetrapeptides are the relative orientation of the four functional groups. In both molecules I and II the <Glu and Arg groups are located at one "end" of the molecule and the Tyr and Trp rings situated at the other end.

Since molecule I exhibits some slight biological LH-RH activity, we should expect to see some similarities occurring between the orientations of the functional groups in molecule I and those (or similar) groups in the native LH-RH conformer. Examination of conformer CC of native LH-RH of paper 1 (see Figures 3 and 4A of this paper) shows an interesting similarity in the relative orientations of the Tyr and Trp rings and also shows the <Glu and Arg side chain of molecule I to be in close correspondence to the <Glu and the C-terminal glycinamide of conformer CC. The similarities are shown further in Figure 4, where several different Trp side-chain conformations of molecule I are shown. In Figure 4B, conformation A of molecule I is given and comparison of the orientation of the <Glu, Tyr, Trp, $-NH_2$ groups of conformer CC (Figure 4A) is seen. Even more striking is the comparison of Figure 4B, in which the A conformer of molecule I is shown where the Trp ring is extended. Because of the flexibility of molecule I, with only small energy differences between low-energy conformers, it seems safe to assume that the pituitary cell receptor site needs a basic group near the <Glu and that the Tyr and Trp rings enhance the fit at the receptor. It is possible that

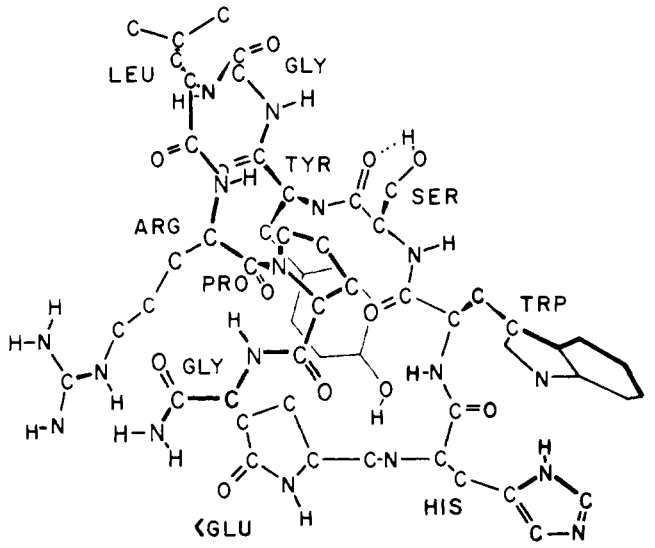

Figure 3. Conformer CC of paper 1.[1] For clarity, some hydrogen atoms have been removed.

the $-NH_2$ group of molecule I acts similarly to the basic His[2] group of native LH-RH, as found in conformer CC. On the other hand, conformer AA of native LH-RH (paper 1) has a Tyr to Trp relationship similar to that in CC, but the basic group located near <Glu is missing.

Figure 4C shows the B and C conformers of molecule I, Table I. Again, the relative orientation of the four side chains is similar to that found for conformer CC of native LH-RH. Only when one goes to conformer D of Table I does this observed orientation change, and it would seem reasonable to assume that one of the three lower energy conformers would be preferred over that of D.

In molecule II (see Figure 2) the relationship between Trp and Tyr is now reversed, when compared to the <Glu-Arg relationship. The <Glu-Arg configuration is nearly identical with that of molecule I; thus, the up-down relationship of Trp and Tyr must be important for biological activity.

Decapeptide Analogues. In the following sections, various amino acid substitutions were made into conformation AA of LH-RH. In each case, three cycles of energy minimization were carried out. All variable dihedral angles of AA, plus any new variable resulting from the substituted amino acid, were included in the energy minimization. The calculations were carried out as described in paper 1.

Ala[6]-LH-RH and Val[6]-LH-RH. It was found that substitution of D-Ala[6] into conformer AA was very favorable and gave an energy of 10.8 kcal/mol lower than the L-Ala[6] conformer. In each case the molecular conformation remained very close to the AA structure. However, it was obvious that in the case of L-Ala[6], a different conformation at the 6 position would be necessary in order to lower the energy. These calculations support the contention that the conformation around the 6 position found here for conformers AA-CC is probably correct.

Calculations similar to that described for Ala[6] were carried out with L-Val[6] and D-Val[6]. As before, the results gave the D residue favored by 11.5 kcal/mol. The added variable for energy minimization was χ_6^1 of Val[6]. The results found here for amino acid substitution at the 6 position are in agreement with the results of studies of analogues by biological activity tests, and the comparison of the calculated and experimental results will be presented in the section on structure-activity relationships.

Ala[9]-LH-RH. Substitution of L-Ala for L-Pro at position 9 was studied. Several starting conformations for the back-

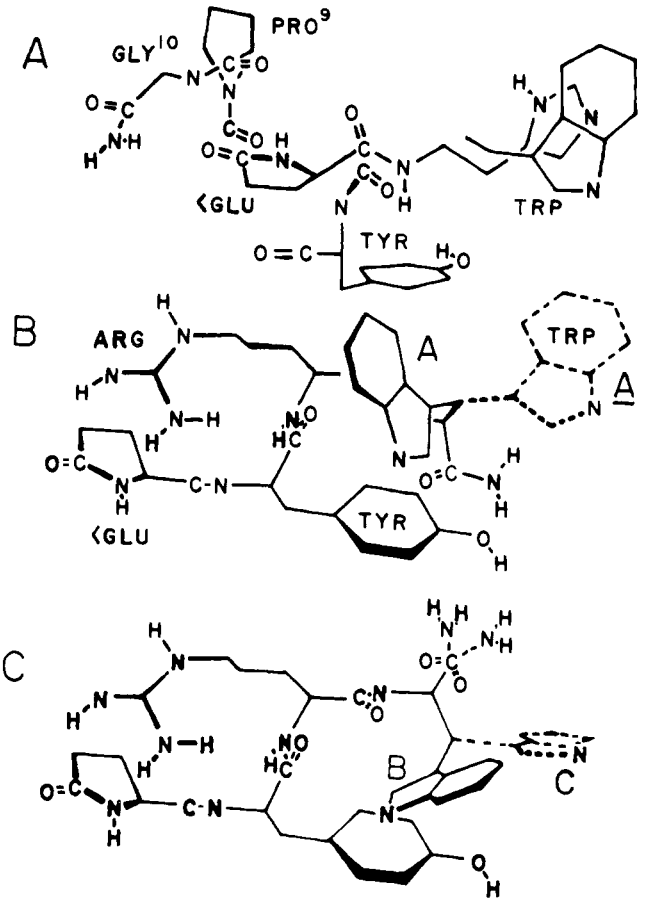

Figure 4. A, relative orientation of the <Glu[1], His[2], Trp[3], Tyr[5], and Gly-NH_2[10] groups of conformer CC of paper 1, with the "ring up" Trp conformation. B, conformers A and A of molecule I. The dihedral angles of A are $\chi_4^1 = -168°$ and $\chi_4^2 = -105°$. The energy difference (ΔE) between A and A is 2.4 kcal/mol where $\Delta E = E_{\underline{A}} - E_A$. C, conformers B and C of molecule I. The dashed lines are conformer C.

bone dihedral angles ϕ and ψ of Ala[9] were taken, using all other dihedral angles of conformer AA of paper 1. Three cycles of energy minimization for all 40 variables were carried out. The results showed that the α_R conformer ($\phi_9 = -61°$; $\psi_9 = -38°$) was ~9 kcal/mol lower in energy than the β conformer ($\phi_9 = -120°$; $\psi_9 = 148°$). The dihedral angles of the remaining part of the molecule moved only slightly from the starting values. This result is significant in that the ϕ and ψ values for the puckered conformation at Pro[9] (i.e., $\phi_9 = -75°$; $\psi_9 = -17.5°$ for AA and $\psi_9 = -28.5°$ for CC) are of lower energy than the extended BB structure and are not significantly different from the α_R conformer found above. This result thus enhances our contention that the puckered Pro[9] is probably the active conformation.

In all the cases studied above, the results for conformer AA would also be applicable to conformer CC, since no interference effects due to the conformer change at His[2] would be expected. It is of interest to note than an experimental verification of the conformation around Pro[9] could be carried out. For example, N-methylation of the Gly[10] peptide nitrogen (or *N*-Me-*N*-Et-des-Gly[10]-NH_2) would sterically eliminate the conformer with Pro[9] puckered (i.e., $\psi_9 = -28.5°$) since only the extended tail ($\psi_9 = 173°$) would be allowed. Activity test should be able to critically distinguish the active form at this position.

Structure-Activity Relationships. Many analogues of LH-RH have recently been synthesized and examined for biological activity and, in general, these studies have shown that amino acid substitution at most positions in the chain

causes a loss in activity. Fragmentation or shortening of the amino acid chain has also shown reduction or loss of activity. In light of the conformers for LH-RH proposed here, a comparison of the experimental data with the calculated structures is essential. The comparisons will be made for each residue, starting at the <Glu residue.

1. <Glu. Deletion of the <Glu ring to yield a free carboxylic acid eliminates the biological activity.[6,7] In conformer CC, a carboxylic group at the <Glu site could strongly change the basic character of this region, leading to reduced binding to the receptor but not necessitating a conformational change. Substitution of Ac-Gly^1, Gly^1, formyl-Gly^1, propionyl-Gly^1, and Ac-Ala^1 all showed extremely low activity.[8] These results probably imply the need for the cyclic carbonyl amide at the N terminus, but the conformational flexibility of Gly and Ala at the 1 position would be very different from the cyclic ring and would change the conformation around this region of the structure.

2. His^2. Substitution and modification of histidine in position 2 has also been shown to lead to low activity,[9-12] as has deletion of His^2. Examination of conformer CC shows that the major conformational change that deletion of His^2 would invoke is to move the <Glu^1 group close to Trp, in a position similar to the <Glu^1 position in conformer AA (see paper 1). The modification of His^2 to 3-Me-His^2 gave ~1% activity.[9] Model CC would allow methylation at the 3 position of the His ring but would not change conformation. On the other hand, methylation would cause steric problems in conformer AA, probably causing a change in conformation. Most probably, the 3-Me-His^2 interferes with efficient binding to the receptor. [Des-His^2,des-Gly^{10}]-LH-RH ethylamide acts as an inhibitor of LH-RH,[13,14] and, as noted above, the remaining chain would not need to change conformation for these modifications.

3. Trp^3. The replacement of Trp^3 by other amino acids indicates that an aromatic residue is necessary at position 3;[10,15,16] also, [des-Trp^3]-LH-RH is not active.[17] Deletion of Trp^3 would cause considerable disruption in the conformers calculated here. However, substitution of other aromatic residues such as pentamethylphenyl-3-alanine-LH-RH was shown to have significant activity (i.e., ~34-70% of native LH-RH).[16] The conformation of Trp^3 in structure CC is such that the pentamethylphenyl group would be allowed, without change in the backbone ϕ_3, ψ_3 values calculated here. The molecules (5-F-Trp)^3, (p-NO_2-Phe)^3, (p-NH_2-Phe)^3, and His^3-LH-RH have very low releasing activities,[16] which are probably related to electronic effects rather than conformational changes,[16] since all of these side chains will substitute for Trp^3 in structure CC, without inducing a change in the backbone conformation.

4. Ser^4. Substitutions at position 4[15,18-20] showed that the hydroxyl group was not essential for activity, but [Ser(t-Bu)^4]-LH-RH and [Leu^4]-LH-RH showed no significant activity while Ala^4 was slightly active.[19,20] In structure CC (or AA), the Ser^4 hydroxyl is surface exposed, and no conformational change would be expected for Ala^4 replacement. However, substitution of a bulky t-butyl group would be expected to have a significant effect on the conformation. The conformational effect of Leu^4 is not so easily identified. Lack of activity for this analogue could be due to a conformational change, but more likely the addition of the hydrophobic side chain has significantly modified the surface properties of this part of the molecule.

5. Tyr^5. Inverting the sequence to [Tyr^3,Trp^5]-LH-RH gave very low activity.[12,21] The hydroxyl group at Tyr^5 was also found to be unimportant for activity.[17] Structure CC would allow the sequence (Tyr^3,Trp^5] with only minor (side-chain) conformational changes, but this is not to say that the same conformer would still be of lowest energy.

6. Gly^6. Position 6 of LH-RH is a key position in the chain, in that its conformation is such that several unique substitutions enhance activity. For example, substitution by L-amino acids leads to decreased activity,[15,19] while substitution of D-Ala^6 or D-Val^6 for Gly^6 enhances the activity to values greater than native LH-RH.[22-24] The D-Ala^6 side chain is favored in the structure presented here by placing Gly^6 in the C_7^{ax} conformation (i.e., C_7^{ax} for a D residue is a low-energy dipeptide conformation equivalent to an L-Ala residue in the C_7^{eq} conformation). The freedom for opening the chain at the 6 position would also be reduced for D-Ala^6 (i.e., noted by a smaller low-energy region on a figure equivalent to that of Figure 5 of paper 1), and the effect of this reduction in freedom would be to enhance the population of the active conformer, thus enhancing the activity. The observation that D-Val^6 retains considerable activity (~30° relative to native LH-RH)[22] is also in agreement with the low-energy conformers found here, although it would be expected that the D-Val^6 would tend to have a higher population of conformers with an extended conformation for ϕ_6 and ψ_6, similarly to the dipeptide results on L-Val^4. The proposed structure would not fit D-Pro^6 for Gly^6 as favorably as D-Ala^6 (in agreement with analogue studies)[22] even though the ϕ_6 value of +80° of conformers AA and CC is very clo to the ϕ = +75° given for D-Pro residues. The reason for the disagreement is that the ψ_6 value of Gly^6 (i.e., −74.8° in AA) is not a low-energy value for D-Pro, and this difference in ψ values would cause a significant conformational change.

7. Leu^7. One of the most significant analogues of LH-RH, with respect to conformation, was the substituted [(N^α-Me]Leu^7]-LH-RH analogue.[25] The effect of this substitution is to eliminate the possibility of forming a hydrogen bond between the N-H of Leu^7 and any other C=O. In particular, the proposed β-II type bend with the C=O of Ser^4 as the acceptor group would be disallowed. The biological activity of this analogue was found to be ~100% of native LH-RH,[25] and, further, [D-Ala^6,(N^α-Me)Leu^7]-LH-RH had ~560% activity relative to native LH-RH. In the structure proposed here (i.e., CC or AA), the methylation of the Leu^7 amide nitrogen is *allowed* with no major conformational changes being necessary. Further, D-Ala^6 will not sterically interfere with the N^α-methyl since they point in opposite directions to one another. The agreement of the [(N^α-Me)Leu^7] analogue with the structure proposed here is very encouraging. However, one further analogue of this type might also be of interest. Examination of model CC indicates that the analogue [(N^α-Me)Arg^8]-LH-RH should also be allowed with only slight conformational change.

Other analogue substitutions at the 7 position include [D-Leu^7]-LH-RH,[10] of 1% potency. This analogue would not be favorable in structure CC (or AA). Further, [Gly^7], [Ala^7], [Val^7], [Ile^7], and [Nle^7] have been shown[9] to have activities, relative to native LH-RH, of ~3, 5, 16, 45, and 30% respectively. Since all of these residues *could* take up the backbone conformation found here for Leu^7, the difference in activities is most probably a consequence of the binding of the hormone to its receptor.

8. Arg^8. The replacement of Arg^8 by Lys leads to a less active LH-RH molecule but retains ~11-28% activity.[9] D-Arg^8 is nearly inactive[10] and would not be allowed conformationally in structure CC. [Gln^8]- and [Leu^8]-LH-RH have low (5 and 1%, respectively) LH-RH activity,[26] indicating a preference for a basic group in this region of the molecule. Both residues would be allowed in structure CC.

9. Pro^9. Substitution of Ala^9 for Pro^9-LH-RH resulted in ~17% LH-RH activity. The results of the calculations for this substitution indicated that the α_R conformation,

found to be of lower minimized energy than the β conformer, would also mimic fairly closely the ϕ^9, ψ^9 proline (puckered) conformation. Transposition of residues 8 and 9 (i.e., [Pro^8]-, [Arg^9]-LH-RH) also gave very low hormonal activities,[26] as would be expected from structure CC presented here.

10. Gly^{10}. Substitutions at the 10 position include [Ala^{10}]-LH-RH,[9] which showed ~10% hormonal activity, and several des-Gly^{10} analogues,[23,24] some of which showed exceptional activity. For example, des-Gly^{10}-[D-Ala^6]-LH-RH ethylamide was found to be ca. five times more active than native LH-RH.[23] [Des-Gly-NH$_2$^{10},Pro-propylamide^9]- and [des-Gly-NH$_2$^{10},Pro-ethanolamide^9]-LH-RH also showed activity much higher than native LH-RH,[27] whereas the Pro-*n*-butylamide^9 analogue was only very weakly active. The analogues noted above are all acceptable into structure CC without major conformational change; thus, the larger alkylamide chain must interfere with binding of the molecule at the receptor. On the other hand, analogues such as Pro^{10}-NH$_2$ and [des-Gly-NH$_2$^{10},Pro-piperidinamide^9]-LH-RH, which are only very slightly active,[27] would necessarily cause a conformational change in structure CC at ψ^9, which in turn could easily disrupt the backbone integrity of the complete LH-RH molecule.

Conclusion

The analogue data described above shows that in every case where a conformational change would occur in structure CC, upon amino acid substitution, the biological activity was shown to decrease dramatically. In several cases, the activity decreased upon substitution, but a conformational change was not indicated from analysis of structure CC. No conclusion concerning the conformation can be drawn from these cases, since the decrease may arise from unfavorable interactions at the receptor site. Clearly, a key experiment would be to find an analogue which would not fit into structure CC without definite backbone conformational changes. In that case, if such an analogue exists, the conformation calculated here would become doubtful. To date, the author knows of no such case.

The calculations used to obtain the low-energy structures presented here have been shown to give conformations in excellent agreement with experimental data for most dipeptides.[4] In the previous studies, conformer populations in nonpolar solvents for both hydrogen-bonded and extended structures were correctly predicted.[4] The dipeptide studies[4] support the calculations presented here and indicate that any conformational changes which might occur upon change of solvent should be minor and result in only side-chain orientations. Since in the biological system the environment at the receptor could be polar, nonpolar, or some complex combination of polar and hydrophobic groups, it is impossible to say what the effect on the conformation of LH-RH the receptor site will exert. One can speculate as to the receptor shape and polarity characteristics, using the surface properties of the LH-RH molecule. For example, from models of structure CC, it can be suggested that the face of the molecule, made up of the Arg^8 side chain, Gly-NH$_2$^{10}, the cis peptide of <Glu^1, and perhaps the His^2 ring, will be in contact with a surface rich in carbonyl, carboxyl, or phosphate groups. The nonpolar surface area of LH-RH, made up of the Pro^9 side chain, Leu^7, and around to Tyr^5, may be necessary to enter into a nonpolar cavity, possibly made up of a larger protein. Further, the Trp^3 side chain may interact through some type of π-π bonding or stack in a plane to plane orientation (similar to the base-base stacking in nucleic acids) with some other aromatic species.

It is clear that the conformation of LH-RH is uniquely suited to allow the molecule to perform its complicated function, and further experiments suggested here should shed additional light on this very interesting biological molecule.

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