Chapter 24. Peptide Conformation and Biological Activity

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The primary event of a hormone-induced physiological response is that of recognition or binding. In order to adequately understand this process, one must know the conformation(s) of the hormone in physiological solution and the conformation(s) when bound to the receptor, presumably on the cell surface. Structure-activity studies of peptides have traditionally ignored conformational effects of chemical modification. Emphasis in this review will be to show how these may be used to distinguish between solution and receptor-bound conformations.

I. Solution Conformation - Nuclear magnetic resonance offers the ability to assign a resonance signal to each nucleus of the peptide molecule under study and interpret the chemical shift, relative intensity, and splitting patterns of these resonance signals in terms of angles and rotational freedom about bonds, and proximity of different groups[1]. Unfortunately, interpretation of results are often clouded by hidden assumptions and technical requirements which are not ideal. For example, exchange broadening of NH resonances at neutral or alkaline pH preclude measurement of J_{NC} coupling constants which yield information of the torsional angle ϕ. Interpretation of coupling constants is complicated by the degeneracy of the Karplus equation as well as by the time-scale of NMR, which renders distinguishing a unique conformer from a rapidly interconverting mixture difficult. These ambiguities may be resolved by measurement of multiple coupling constants especially in peptides enriched with either ^{13}C or ^{15}N, as exemplified with valinomycin[2]. Relaxation measurements offer insight into molecular dynamics but require high concentrations of peptide for natural abundance studies of ^{13}C NMR, leading to aggregation[3,4]. Interpretation of spin-lattice relaxation times in terms of overall rotational correlation times and segmental motions are model dependent[5] and inadequate determinations of correlation times have often been made. Measurements of nuclear Overhauser effects provide a valuable auxillary method for intermolecular distance constraints[6], but correlation times must be determined for rigorous interpretation. Other procedures which offer experimental advantages over NMR, due to lower concentration requirements, are laser Raman spectroscopy, e.g. valinomycin[7], and fluorescence energy transfer, e.g. enkephalin[8] and angiotensin[9] analogs.

Calculation of structures with potential energy minima ignore the observations that the conformation of most peptides is very dependent on solvent, which current computational techniques are inadequate to handle in detail. In addition, a dielectric function appears necessary[10] to adequately predict minima of charged molecules with the semi-empirical approach. Molecular orbital approximations of solvent-solute complexes may prove more realistic[11].

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Considering the conformational lability of peptides in solution, one should not be surprised that interaction with an asymmetric receptor, which may include electrostatic, hydrogen-bonding, and hydrophobic interactions, affects the conformation of the peptide hormone. Evidence exists in studies[12,13] of the binding of S-peptide to S-protein to form ribonuclease-S' that binding of a peptide to a protein induces a conformational change from that observed in solution. Recent studies[14] on luliberin, the hypothalamic decapeptide which controls the release of gonadotropins from the pituitary, have led to the conclusion "that a predetermined solution conformation is not required for biological activity." A similar conclusion was reached[15] based on conformational studies on gramicidin S analogs with equivalent biological activity but with distinctly different conformations.

In summary, examination of the available information indicates that biologically active peptides in solution are flexible with time-averaged conformations which offer little insight into their biological activity.

II. Receptor-bound Conformation - While some workers have argued that the most highly populated conformer in solution will bind with the highest affinity to the receptor, there are excellent *a priori* reasons for believing that this will not be the general case[16], somewhat analogous to the observations that transition state analogs rather than the predominant conformers found in solution bind most tightly to enzymes. It is, therefore, necessary to develop approaches which allow one to deduce the receptor-bound conformation independent of solution observations. Systematic examination of semi-rigid analogs for their conformational effects and correlation with biological activity offers one rational approach. Marshall and Bosshard[17] first showed the dramatic effects of the α-methyl substitution in restricting the conformational flexibility at the substituted residue to a value near those for a right-handed ($\phi = -60$, $\psi = -50°$) or a left-handed ($\phi = 60°$, $\psi = 50°$) α-helix. These calculations have since been confirmed by two other groups[18,19] independently. Two crystal structures of fragments of alamethicin which contain a total of four residues of amino-isobutyric acid, i.e. α-methyl-alanine (Aib), have recently been solved[20,21]. Of the four Aib residues, three occupy the two predicted minima almost exactly ($\phi = -52°$, $\psi = -38°$; $\phi = 48°$, $\psi = 42°$; $\phi = -51°$, $\psi = -46°$) while the fourth is well within the allowed area ($\phi = -72°$, $\psi = -11°$), as shown in Fig. 1. These results confirm the calculations and indicate the strong constraints imposed by such residues when incorporated into peptide chains.

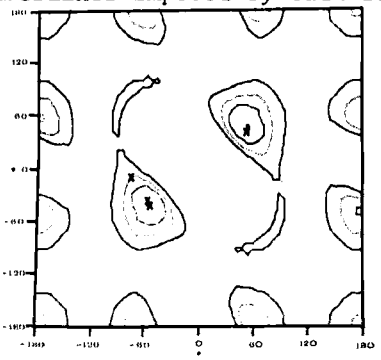


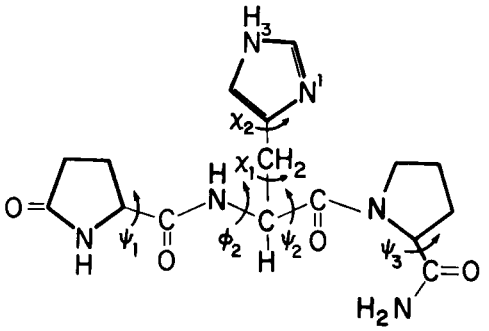
Fig. 1. Potential energy plot for N-acetyl-Aib-methylamide with experimental observations (X) of Aib residues.

The problem of interest is whether these calculated constraints apply to peptides when they bind to receptors. Agreement with the observed values from protein crystallography[22] argues in favor, as one would think that the range of potential distortions of the solution conformation which might occur on binding to the receptor had been adequately sampled in the folding of a large protein. A peptide would be expected to tolerate less distortion than a segment of a protein chain as the end residues can be moved without sacrificing a large number of energetically favorable interactions in order to relieve the local strain.

A basic assumption of a common backbone conformation which orients the essential functional group in the correct spatial positions for all active analogs underlies this approach. Intersection of the set of all possible conformations for each analog contains the biologically active conformer. Identification of essential functional groups by traditional structure-activity studies allows transformation of the problem from conformational space to orientation space where one examines possible pharmacophores, i.e. required groups and their orientation, and removes the objection of a possible analog with a different conformation having activity.

In order to illustrate these concepts, the current state of knowledge of conformation of three peptide hormones will be reviewed.

III. Thyroliberin - To the first level of approximation, six torsional angles are needed to describe the conformation of thyroliberin (TRH), the hypothalamic tripeptide which controls the release of thyroid stimulating hormone from the pituitary: ψ_1, ϕ_2, χ_1, χ_2, ψ_2, and ψ_3.



A. NMR Studies - A conformation for TRH in DMSO solution has been proposed[23] in which there is a hydrogen bond between the trans carboxamide hydrogen and the His-Pro carbonyl oxygen and another hydrogen bond between the imidazole N-1 and the Glp-His peptide bond hydrogen. This latter hydrogen bond is proposed because it stabilizes a structure with $\phi_2 = -150°$, compatible with the measured coupling constant. Double resonance techniques in which the $C_\beta H_a$-$C_\beta H_b$ coupling constants are measured lead to an interpretation that a value of $\chi_1 = +60°$ is slightly preferred, which agrees with the assigned hydrogen bond involving the imidazole ring.

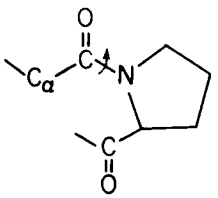
Two groups[24,25] have re-evaluated the conformation of TRH in DMSO by pmr and find that $\chi_1 = +60°$ to have the lowest population. They identify the most highly populated rotamer as $\chi_1 = 180°$ because that is the one in

which the imidazole is placed nearest the proline. They also measure the temperature dependence of the amide hydrogen chemical shifts and find no evidence for hydrogen bonds. They argue that the steric bulk of the histidine and proline force the peptide backbone into an extended conformation ($\phi_2 \simeq -150°$, $\psi_2 \simeq 150°$), which is consistent with the histidine J_{NC} of 7.2 Hz. The anisotropy of the proline δ and carboxamide hydrogens is due only to the proximity of the imidazole.

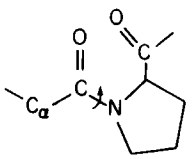
Donzel et al.[26] prepared the analog [MeHis^2]-TRH and reported its biological activity as 115%. This analog cannot possibly form a hydrogen bond involving the imidazole N1. They also studied its pmr spectrum in DMSO and concluded, from similar coupling constant analysis, that the histidine imidazole has no preferred orientation among the three staggered conformers. The rest of the spectrum is very similar to that of the parent compound.

Bellocq and Dubien[27] examined the pmr spectrum of [(3-methylimidazolyl)alanine^2]-TRH, an analog with 800% biological activity. Its spectrum in DMSO agrees very well with that of TRH. The temperature dependences of the chemical shifts for all the amide hydrogens were determined and found to be quite similar for all the hydrogens, implying that none are involved in hydrogen bonds. They suggest the anisotropy of the proline δ and carboxamide hydrogens to be due to the proximity of the imidazole ring. Bellocq et al.[28] also examined the pmr spectrum of [(2-thienyl)alanine^2]-TRH, an analog with 75% biological activity. Its spectrum in DMSO differs from that of TRH in that the non-equivalence of the proline δ hydrogens is abolished and that of the carboxamide hydrogens is reduced. Analysis of the rotamer population about χ_1 gives a distribution of 60% at $\chi_1 = -60°$. Thus, the data seem to indicate an extended conformation in DMSO, without intramolecular hydrogen bonds, determined mostly by the steric bulk of the histidine and proline residues. However, whatever the conformation of TRH in DMSO, it does not seem to be relevant to biological activity.

The pmr spectrum of TRH has been studied in water[25,29]. The overall model derived from these data is similar to the DMSO model. The molecule is in an extended conformation ($\phi_2 \simeq -150°$, $\psi_2 \simeq 155°$) unstabilized by hydrogen bonds and determined mainly by the steric bulk of the histidine and proline residues. There is less interaction between the imidazole and carboxamide groups compared to DMSO solution which may indicate more solvation of these groups in water or a change in the average value of ψ_3.



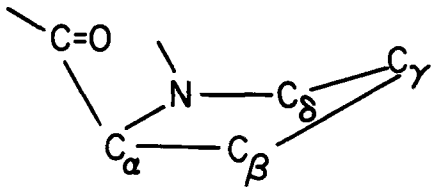
TRANS X-Pro Bond
ω = 180°

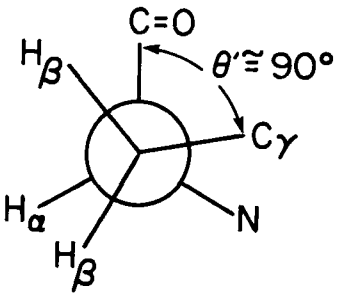


CIS X-Pro Bond
ω = 0°

A number of ^{13}C studies[30,31] have demonstrated the presence of some cis isomer of Pro in TRH by the doubling of the pyrrolidine carbon resonances. The amount of cis isomer, estimated from relative peak heights, is

uniformly reported as some 15-20%. This is in contrast to the behavior of N-acetyl-proline amide and other model X-Pro compounds, where the amount of cis isomer is some 25-40% in D_2O solution. This suggests some steric or conformational preference for a trans His-Pro bond in TRH. Deslauriers et al.[32] have examined the imidazole tautomer ratio in TRH by examining the pH dependence of the histidine carbon chemical shifts in D_2O solution. The behavior of thyroliberin is very similar to that of free histidine, which has been shown to be predominantly the N3-H tautomer. The spin-lattice relaxation times show no change for the imidazole carbons on going from pH 4.9 to pH 9.9, providing evidence against any conformer having a hydrogen bond involving the imidazole as a hydrogen bond acceptor[32,33]. The relaxation times of β, γ, and δ pyrrolidine carbons are appreciably higher than that of the alpha carbon, increasing in the order $\alpha < \beta \approx \delta < \gamma$. This suggests a rapid interconversion between endo and exo forms of the ring. Haar et al.[31] examined the ^{13}C vicinal coupling constants using [85% C-13 enriched proline^3]-TRH and propose the Cδ endo puckered conformer to predominate. It may be that there is a rapid conversion between exo and endo forms as suggested by the relaxation times. In general, the ^{13}C studies in D_2O give the overall impression of a relatively flexible molecule whose conformational mobility is similar to model dipeptides and acylamino acids. There is no evidence for hydrogen bonding.





Cγ ENDO Proline

B. Theoretical Energy Calculations - There have been several attempts to calculate the minimum energy conformation of TRH. The calculated conformers all share one feature: they are stabilized by intramolecular hydrogen bonds. Blagdon et al.[34] find two minimum energy conformers. The global minimum features a "hairpin turn" with the histidine torsional angles ϕ_2 and ψ_2 approximately those of a right-handed alpha helix. There are hydrogen bonds between the imidazole N1 and Glp-His amide hydrogen and between the Glp-His amide carbonyl and the trans carboxamide hydrogen. The other low energy conformer features an extended conformation at the histidine residue. Burgess et al.[35] calculate a number of similar conformers, the lowest energy one being very near the extended conformer of Blagdon et al. There is one hydrogen bond, between the trans carboxamide hydrogen and the His-Pro amide carbonyl. Belle et al.[36] propose two hydrogen bonding schemes, one identical to that of Burgess et al. and, alternatively, one in which the imidazole is in the N1-H tautomer; there is a hydrogen bond between this N1-H and the His-Pro amide carbonyl. It is very diffi-

cult to assess the relevancy of energy calculations especially when the data indicates that TRH is well-solvated and lacks intramolecular hydrogen bonds.

C. Analog data - It is possible to probe the structural and conformational requirements for binding to the TRH receptor by evaluation of the biological activity of analogs of the hormone, in particular, those into which conformational constraints have been introduced. TRH is ideal for such a study because of the very close correlation between measured binding affinity and biological potency[39,40], suggesting that binding and activity are very closely coupled phenomena or at least have the same structural requirements. This is supported by the observation that the few inhibitory compounds known are only active at 10^4 higher concentrations than TRH[39].

There have been a number of reviews[40-43] of structure-activity relationships; the pertinent conclusions are summarized below.

1. The Glp residue: The lactam ring is necessary for good activity. Heteroatoms in the ring or short branches off the ring are compatible with activity. This implies that only the lactam structure is important and that the ring merely orients it. The fair amount of space available at the receptor at this point is emphasized if one considers the carbonyl of [N-formyl-Pro^1]-TRH, an analog with 10% activity, as equivalent to the lactam carbonyl.

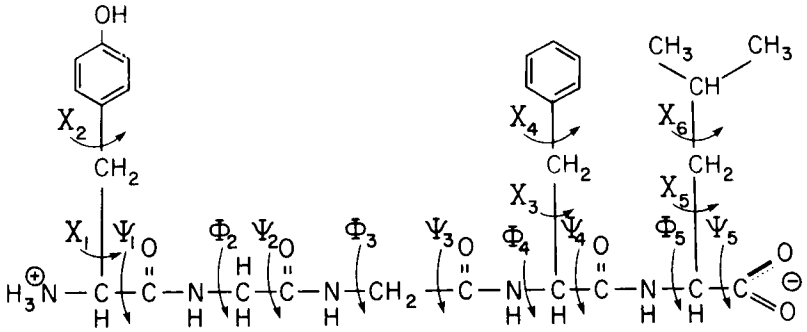
2. The Pro amide residue: Proline monoalkyl amides and prolinol have good activity whereas the free acid is inactive, suggesting the necessity of a hydrogen bond donor in this position. Isosteric rings and MeAla which has conformational constraints similar to Pro, are active, as are the corresponding 4 and 6 membered ring amino acids. The purpose of Pro seems to be to hold the carboxamide in a certain orientation.

3. The His residue: The most active analogs contain a planar, five membered ring side chain with a nucleophile in the δ position. The activity of Phe, Leu, and cyclohexylalanine in this position, however, suggest that this is not an absolute requirement for binding and that only a branched amino acid is strictly required.

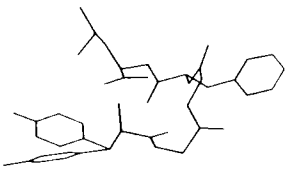
As has been noted, substitution of a methyl group for a backbone hydrogen in a peptide imposes severe conformational constraints. In particular, residues preceding an N-methyl residue are restricted to basically the extended conformation region ($-180° < \phi < -60°$, $+60° < \psi < +180°$), which puts limits on the angles ϕ_2 and ψ_2 of TRH. Similarily, since [MeHis^2]-TRH is an equipotent analog, it implies that ψ_1 is similarily constrained. [D,L-Glp(αMe)^1]-TRH is an analog with 50% activity[43]. Since [D-Glp^1]-TRH has only .02% activity, it is reasonable to assume that the L-α-methyl isomer is equipotent with TRH. When one considers the overlap in conformational space of the N-methyl and α-methyl analogs, one finds a small region of overlap, $+60° < \psi_1 < +120°$. This angle could be refined further by considering [Glp(αMe)^1, MeHis^2]-TRH. Its region of overlap with the two monosubstituted analogs is minute, $\psi_1 = +61°$. If the disubstituted analog is inactive, this implies that the methyl groups must be forced to occupy the same space when the molecule is bound to the receptor, which implies $\psi_1 \approx +120°$. Using such conformationally restricted analogs, work is in progress to refine the receptor-bound conformation of

TRH.

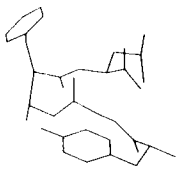
IV. Enkephalin - An intense interest exists in the smallest of the opioid-like neuropeptides having the sequences Tyr-Gly-Gly-Phe-X, where X is either Leu (LENK) or Met (MENK). The existence of rigid opiates which interact with the same receptor offers a unique opportunity for deducing the pharmacophore and the receptor-bound conformation.



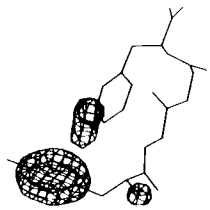
A. Crystal Structure - X-ray crystallographic structures have been reported for LENK[44] (Fig.6a) and the enkephalin fragments Tyr-Gly-Gly-Phe (TGGP)[45], Gly-Gly-Phe-Leu (GGPL)[45], and Tyr-Gly-Gly (TGG)[46]. The hydrogen bonding scheme and β-bend seen in the crystal of LENK is different from any proposed by theoretical calculations or solution NMR. Two points argue against the relevance of the crystal structure of LENK to biological activity. First, the tyrosine residue occurs in two conformers neither of which corresponds to the phenolic ring and nitrogen orientation seen in opiates. In addition, the backbone conformation has a hydrogen bond involving the amide hydrogen of Phe^4 and the carbonyl oxygen of $_2Tyr^1$, which would be incompatible with the exceptional activity of [$D\text{-}Ala^2$, $N\text{-}Me\text{-}Phe^4$]-LENK[47]. The crystal structure of the fragment TGGP has hydrogen bonding analogous to the pentapeptide but the orientation of the backbone and the side chains of Tyr^1 and Phe^4 are quite different. The other fragments of enkephalin have no intramolecular hydrogen bonding. For small, linear molecules with low internal barriers to rotation, intermolecular packing forces can dominate intramolecular forces[48], making correlation between solution or receptor conformation with solid state structures tenuous.



(a) Crystal structure of LENK[44]



(b) Theoretical Conformation of Isogai et al.[56]



(c) Receptor-bond conformer and morphine pharmacophore[72].

B. NMR Studies - Numerous proton studies[47,49-54] of MENK as well as ^{13}C NMR studies[50-53], have been reported; limited studies[50,54,4] on LENK indi-

cate only minor differences. A number of discrepancies in the early studies have been resolved by noting that the zwitterionic form is compatible with a 2-5 βI turn in DMSO[50-51] while the cationic form exhibits a different conformation[51,54] not so well defined. These observations are complicated by the report[4] of association of enkephalin in a variety of solvents. The NT_1 values for the α-carbons of LENK in DMSO are very similar[50] which is compatible with an antiparallel β-dimer as proposed for the aggregated state[4]. In TGGP, the minimal fragment showing enkephalin activity, a 1-4 β-turn has been suggested[45]. [D-Ala^2]-MENK exhibits almost identical spectra as the parent compound and analysis of the C_α-C_β rotamer populations for both peptides shows significant populations for each rotamer[52].

C. Other Physical Techniques - Schiller and co-workers[8,55] have reported that [Trp^4]-MENK has the same affinity for the opiate receptor in rat brain as MENK. Examination of the intramolecular energy transfer between the Tyr^1 and the Trp^4 indicate an average separation of 9.3 Å either at pH 1.5 or pH 5.5. Since the concentration was only 3×10^{-5} M, one assumes the differences seen by NMR at these two pH values may reflect changes in aggregation. Furthermore, the observed phenol fluorescence in both aqueous solution and butanol rules out the proposed hydrogen bonded structures[4,56] which utilize the phenolic hydroxyl. The distance of 9.3 Å is supportive of a folded structure.

D. Theoretical Energy Calculations - Enkephalins have a minimum of seventeen variables, i.e. the rotatable bonds, if the amide unit is assumed planar and bond angles invariant. A 20° incremental systematic search would require analysis of about 10^{22} conformers. As a consequence, all reported semi-empirical energy calculations on enkephalin performed a limited exploration of conformational space[56-59]. Isogai et al.[56] found the most favorable conformer (Fig. 6b) to be a β-II' bend stabilized by a hydrogen bond between the phenolic hydroxyl of Tyr^1 and the carbonyl oxygen of Phe^4 which is not seen in aqueous solution[55]. The energetically most favored conformers are incompatible with the observation that [D-Ala^2]-MENK retains activity[60] while a large decrease in binding and activity accompanies the [L-Ala^2] analog[61] and thus the theoretically predicted structures must have little in common with the receptor-bound conformation. Momany[58] examined the lowest energy conformers of the charged and C-terminal amide forms of analogs of MENK. The conformation selected as being compatible with the active analogs examined has little structural correspondence with morphine. This conformation also conflicts with the observations that [Aib^2]-MENK and [L-Phe(αMe)^4]-LENK[62] are active and D-Ala^3 or L-Ala^3 analogs are inactive[63,64]. De Coen et al.[59] investigated the zwitterionic forms of MENK and LENK and three analogs: D-Ala^2, D-Ala^3, and D-Phe^4. A backbone conformation was selected which is not an energy minimum for the native enkephalins but is the minimum energy state for [D-Ala^2]-MENK and is not shared by the inactive [D-Ala^3] or [D-Phe^4] analogs. Strictly speaking, this conformer would contradict SAR data by predicting [Aib^2]-MENK and [L-Phe(αMe)^4]-LENK to be inactive. Because of the coarseness of the torsional scan, a ± 20° shift of predicted backbone angles could accommodate known structure-activity relationship (SAR) data. The side chain torsion angles of Tyr^1 proposed do not position the nitro-

gen and the phenolic ring in enkephalin in the same orientation as the tyramine moiety seen in most opiate compounds.

E. Receptor-Bound Conformation - Numerous analogs of enkephalin have been prepared[60-65] many of which contain substitutions with conformational information, e.g. D-amino acids. Beddell et al.[66] have examined the compatibility of active analogs containing such substitutions with particular conformations of the peptide backbone which are stabilized by intramolecular hydrogen bonds and previously characterized. Only 11 of the 26 possibilities considered were found to be consistent with the SAR data. Structures involving hydrogen bonding to the amide bond between residues 4 and 5 such as suggested by NMR can also be eliminated as essential due to the activity of analogs in which Phe^4 is replaced[67,68] with phenethylamine, eliminating the carboxamide group along with residue 5. Other highly active analogs with conformational constraints which must be considered are [Phe(αMe)^4]-LENK[62] and [D-Ala^2, $MePhe^4$]-LENK[47].

An alternative approach has been to assume a pharmacophore for opiate activity and a correspondence between enkephalin and the essential groups of this pharmacophore. Correspondence between the tyramine moiety of morphine and Tyr^1 has been suggested[69-72]. Several investigations[70,71] have related the aromatic ring of Phe^4 with the 19-phenethyl substituent on 7-(1-phenyl-3-hydroxybutyl-3)-endoethenotetrahydrothebaine (PET). Using 5-phenylbenzomorphan as a model, Gorin and Marshall[72] assumed a correspondence between the aromatic ring of Phe^4 and atoms C5 and C6 of morphine's non-aromatic C-ring. The activity of analogs in which Phe^4 has been replaced by cyclohexylalanine[68] or by carboranylalanine[73] indicates that aromaticity of Phe^4 is not essential. Assuming the correspondence of the conformation of Tyr^1 to that observed in morphine (χ_1 = 197°, χ_2 = -106°), conformational space was systematically explored at 31° torsional increments to determine those conformations in which the assumed correspondence between Phe^4 and the C-ring was possible. A single conformer (Fig. 6c) was found which appears consistent with the published SAR data[62,68].

V. Angiotensin - Of the eight amino acid residues, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, comprising angiotensin (AII), Val^3-Phe^8 is the minimal unit consistent with binding and activity. SAR studies[74,75] emphasize the importance of Tyr^4, His^6 and Phe^8. Single substitution of Tyr^4 and Phe^8 have led to partial agonists which have been used as competitive antagonists. The role of the C-terminal carboxylate has been reinvestigated[76] and may be essential to recognition, as analogs incapable of biotransformation to a free carboxyl in this position are devoid of activity. The other groups appear important for correct positioning of these functional groups and increased affinity.

A. Solution Studies - The combined results of the physical techniques used to study the solution conformation of [Asn^1, Val^5]-AII agree[22] that this linear octapeptide has a time-averaged preferred solution conformation. Proton NMR indicates two conformational changes, one with pKa of 5.5 and the other with pKa of 10.2. The first conformational change would

appear associated with the titration of either the N-terminal amino group (pKa = 6.98), or the imidazole of His-6 (pKa = 6.26), or more likely both. The second is associated with ionization of the phenolic hydroxyl of Tyr-4. Measurement and assignment of the NH-CαH vicinal coupling constants limit the possible values for the torsional rotations about the amide to α-carbon bond. Exchange experiments indicate that Val-3, Val-5, and possibly Tyr-4 amide protons are either involved in intramolecular hydrogen bonds or sterically shielded from the solvent. Titration data[77] indicate that the N- and C-terminal ends of AII are in closer proximity then would be expected in a random coil. Carbon-13 spin-lattice relaxation studies[78] indicate that the α-carbons of the peptide backbone are approximately equally restricted in their motion. More recent investigation[3] of T_1's of AII as a function of field strength indicate overall motion (τ_R) of the molecule of approximately 6×10^{-9} sec which implies aggregation at pH 4.3 where these studies were done. This observation raises serious questions regarding the interpretation of data gathered previously on AII at this pH and concentration. The conformational change seen on going to alkaline pH as well as the presence[79] of cis-Pro at pH above 8 may simply imply a change in aggregation. Unfortunately, the question of aggregation will require re-examination of all the physical data obtained on angiotensin at higher concentration. The fluorescence energy transfer data gathered[9] at 10^{-5} M on [Trp^1, Val^5]-AII suggests close proximity (< 8 Å) of residue one and four and may be relevant to the monomeric state.

B. Receptor-Bound Conformation - By a variety of analogs with conformational constraints, the approximate values for the torsional rotations about the NH-CαH bond of residues 3, 4, and 5 when bound to the receptor have been deduced. In the case of position 3 and 5, the arguments were based on the high activity retained by substitution of the cyclic residue Pro at these positions. The constraint on position 4 is much better defined as [Phe(αMe)^4]-AII has the same activity at [Phe^4]-AII which implies that an α-methyl substitution at position 4 is fully compatible with biological activity[80]. Based on analysis of other analogs of AII which restrict conformational freedom, further constraints of the receptor-bound conformation have been proposed. While comparison of these values for ϕ with the values derived from examination of the NH-CH coupling constants has been made, two difficulties preclude a firm conclusion. First, the measurement of the coupling constants must be done at acid pH; and second, the state of aggregation of angiotensin under physiological conditions would appear different than that during most physical studies.

IV. Conclusion - The data currently available suggest strongly that the predominate solution conformation does not directly correspond to the biologically active conformer. Receptors either induce the conformation required for recognition and activation or select a minor conformer from the ensemble available in solution.

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