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Folded Conformation of an Immunostimulating Tetrapeptide Rigin: High Temperature Molecular Dynamics Simulation Study

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Abstract—Employing high temperature quenched molecular dynamics (QMD) simulations the conformational energy space of an immunostimulating tetrapeptide rigin: H-Gly^{341}-Gln-Pro-Arg^{344}-OH, is explored. Using distance dependent dielectric ($\varepsilon = r_{ij}$) 31 different low energy starting structures with identical sequence were computed for their conformational preferences. According to the hypothesis of O'Connors et al. [*J. Med. Chem.* 35 (1992), 2870], 83 low-energy conformers resulted from unrestrained molecular dynamics (MD) simulations, could be classified into two energy minimized families: **A** and **B**, comprised of 64 (Pro C$^{\gamma}$-*endo* orientation) and 19 (Pro C$^{\gamma}$-*exo* orientation) structures, respectively. An examination of these families revealed the existence of a remarkably similar *folded* backbone conformation: torsion angles being $\phi_{i+1} \approx -65°$, $\psi_{i+1} \approx -65°$, $\phi_{i+2} \approx -65°$, $\psi_{i+2} \approx -60°$, characterizing a *distorted* type III β-turn structure across the central Gln-Pro segment. The *folded* conformation of rigin is devoid of a classical 1 ← 4 intra-molecular hydrogen bond nevertheless, the conformation is stabilized by an effective '*salt-bridge*', i.e., Gly $H_3N^+ \ldots C^{\alpha}OO^-$ Arg interaction. Surprisingly, in both the families the *unusual* folded side-chain dispositions of the Gln residue favor the formation of a *unique* intra-residue 'main-chain to side-chain' H-bond, i.e., N^{α}–$H \ldots N^{\varepsilon}$ interaction, encompassing a seven-membered ring motif. The conformational attributes may be valuable in de novo construction of structure-based drug candidates having sufficient stimulating activity.

Introduction

An immunostimulating linear tetrapeptide rigin: H-Gly^{341}-Gln-Pro-Arg^{344}-OH, located in the Fc portion and connects the CH2 and CH3 domains of human immunoglobulin G molecule, is structurally as well as functionally related to an extensively studied tuftsin molecule: H-Thr^{289}-Lys-Pro-Arg^{292}-OH. The marked therapeutic potential of these immunostimulating tetrapeptide.[1–3] have been reviewed by many investigators. Veretennikova et al. reported that the overall phagocytosis stimulating activity of rigin is same as for the tuftsin.[3] In general, the peptide immunomodulators are expected to generate specific physiological response in the target cells via their specific surface receptor and it is plausible that the rigin may activate macrophages through binding to its own specific receptor.[4]

In an attempt to develop more potent analogues Rocchi et al. attempted the chemical synthesis and bio-activity evaluations of glycosylated rigin.[5,6] The fact that these glycosylated derivatives failed to exhibit any appreciable phagocytosis stimulating activity point towards that the precise knowledge of the three-dimensional structure may be prerequisite for the structure-based design of potent analogues. Undoubted therapeutic usefulness of the immunostimulating agents in various diseases including antimicrobial, antiviral and antitumour activities, suggests that a systematic conformational exploration of an equipotent tetrapeptide rigin is desirable.[1,2,7] In an effort to get insight into the preferred conformation(s) of the tetrapeptide, in a preliminary investigation we employed a combination of theoretical: unrestrained MD simulations in *implicit* dimethylsulfoxide (DMSO) and experimental: 1D and 2D ^{1}H NMR spectroscopic methods in DMSO-d_6 and proposed that in a polar environment, an *unusual* type VII β-turn structure may be predominant.[8]

Over the decades, computer-assisted molecular modeling of a variety of biologically active peptides has witnessed the success of the structure-based drug design.[7,9–12] It should be emphasized that theoretically derived structural informations can present strong self-consistent evidence

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for the well defined conformational features needed for modeling which in turn may be exploited for rational designing and rigidification.[9–13] Of particular interest is the computer-assisted molecular modeling of a closely related tuftsin molecule[14–21] under low dielectric constant conditions ($\varepsilon = 3.5$) which played a key role in providing valuable structural informations that eventually enabled the design of superactive tuftsin derivatives.[1,19]

The decision to pursue an exploration of potentially available conformational space of rigin sequence, employing unrestrained MD simulations protocol in vacuo conditions, i.e., distance dependent dielectric of ($\varepsilon = r_{ij}$), is primarily relied on success of theoretical energy calculations under effective low dielectric constants by Konopinska et al.[15] and Nikiforovich et al.[16,17] and its subsequent experimental validation by Chipens et al.[59] of the tuftsin molecule. Realizing the importance of charges on the preferred low energy conformation and presumably to influence the receptor environment, the characterization of energetically preferred molecular structures may represent a plausible starting 'model' for the bio-active conformation of rigin, presumably for structure-based design of the pharmacophore.

Experimental

Search for the starting structures

A zwitterionic structure of rigin, comprised of L-amino acids (Fig. 1) with standard parameters for an all-atom system, was considered for mapping the conformational space. First the systematic grid search was carried out by employing a dielectric constant of 2 and atom–atom potentials with force-field parameters reported by Momany et al. using the CONFD program.[22] Ten internal torsional angles: ψ_1, ϕ_2, ψ_2, χ_2^1, χ_2^2, ψ_3, ϕ_4, χ_4^1, χ_4^2 and χ_4^3 were allowed to vary at 20° resolution while ϕ_3 of the pyrrolidine ring and ω of the peptide bonds were constrained at −65 and 180°, respectively. The search for the allowed conformational space was performed from N- to C-terminal and vice versa. Of the 3,779,136 (2×18^5) backbone structures screened, eight low energy structures, within 6 kcal/mol CONFD energy, from the most stable one having distinct backbone conformations were short listed for side-chain optimization. The side-chain conformation optimization also involved a grid search of the torsion angles: χ_2^1, χ_2^2, χ_4^1, χ_4^2 and χ_4^3, at 20° resolution from both the N- to C- and C- to N-termini. The complete optimization yielded 15 most stable structures which were selected for MD simulations.

For the initial optimization of the dynamics parameters, a fully-extended conformation ($\phi = \psi = \omega = \chi = 180°$) of the rigin was considered. In addition, thirteen rigin structures extracted from Brookhaven Protein Databank, whose crystal structures have been reported to high resolution ($R \leq 2.0$ Å), the accession numbers: 1CAU, 1CAV, 1CAW, 3TGL, 4TGL, 1FRT, 1FCC, 1FC2, 1MCO, 1FC1 (chain A and B) and 1CAX (chain B and F), were considered to generate starting structures for minimization. Moreover, two proteins: (1AWE; RMSD 2.3 Å and 1PMS; RMSD 1.24±0.35 Å), whose three-dimensional structure have been determined from high resolution NMR, were also included as starting structures.

MD simulation studies

All the unrestrained MD simulations were performed on DEC ALPHA 3000 workstation with AMBER4.0 package using the all-atom standard parameters with *trans* amide bonds.[23] During simulations at high temperature, an additional torsional restraint of 5 kcal/rad^2/Å^2 was added uniformly to the peptide bonds in order to minimize the *trans–cis* interconversion.[24] The dynamics parameters and protocols were uniformly employed to all the 31 different starting structures (data not shown) for the high temperature molecular dynamics calculations. A distance dependent dielectric continuum ($\varepsilon = r_{ij}$ in the Coulombic term) have been used.[25–28] It should be noted that while the packing effects in the proximity of the receptor can be crucial, the structures

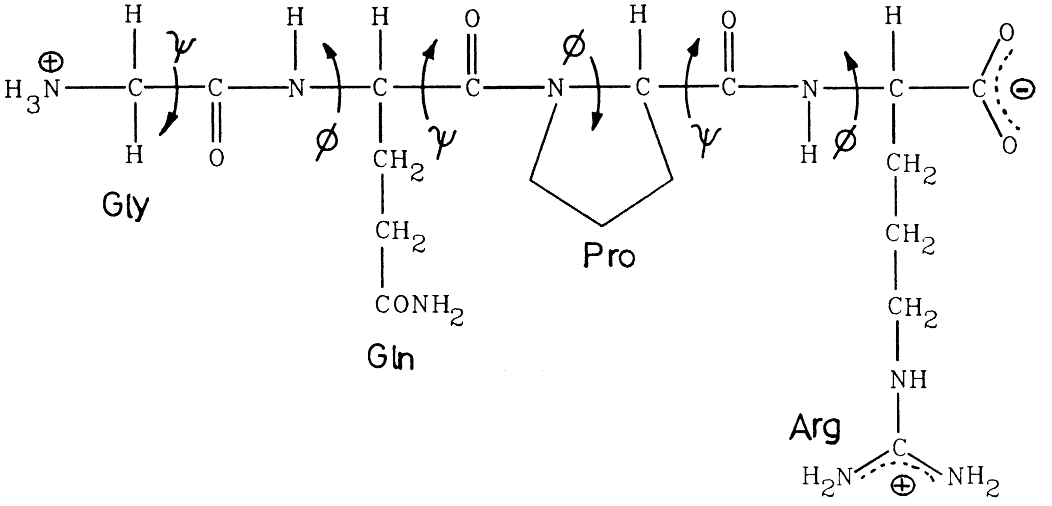


Figure 1. Schematic representation of the zwitterionic chemical structure of the rigin molecule. The relevant backbone torsion angles (ϕ, ψ) are indicated by arrows.

representing the low energy tail of the Gaussian-like distribution of energies are expected to be the most probable conformations under the dielectric continuum employed.[19] It has been pointed out that the dominant influence on the potential energy of the structures could be due to dielectric screening.[19,29,30] The advantages of such an approach to understand the structure-function relationship of low molecular weight biologically relevant peptides has been highlighted.[1,7]

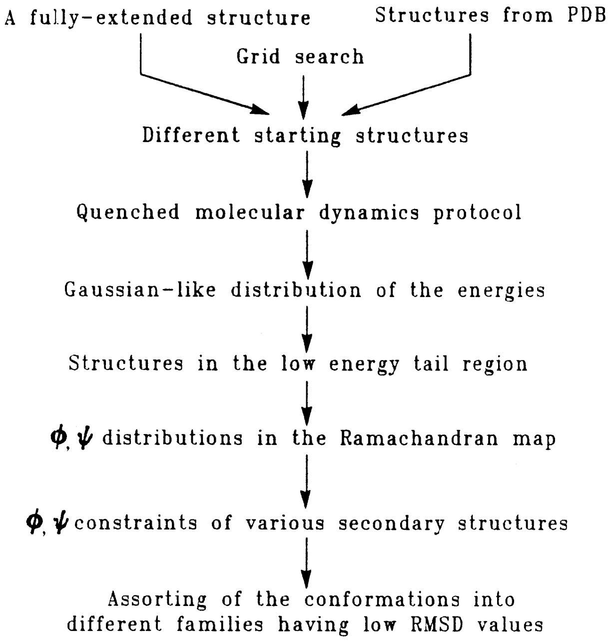
For quenched MD calculations, the coordinates of each starting conformation were first minimized and then heated gradually to 900 K over 5 ps of dynamics using Berendsen coupling scheme.[31] At high temperature, the molecular dynamics production runs were performed with a time step of 1 fs for a total time of 100 ps employing Verlet's algorithm[32] with Leap Frog approximation by Hockney and Eastwood.[33] The trajectories for each set were quenched (minimized by freezing) by lowering the temperature to 0 K at every 5 ps of dynamics. The energy minimization procedures involved 500 steps of steepest-descent gradient with initial step length of 0.01, followed by 1000 steps of conjugate gradient, converging when the energy difference for successive steps is <0.0001 kcal/mol/Å. Finally, the minimized coordinates of 20 structures, resulted from each simulation, at 0 K were equilibrated at 300 K for 5 ps and then further energy minimized for obtaining the relevant structural informations at ambient temperature. An overall approach employed for the conformational analysis i.e., computer assisted molecular modeling, of rigin is depicted in Scheme 1. We also believe that the present molecular modeling protocol can further be extended successfully to other 'conformationally flexible' bio-active peptides and peptidomimetics.

Results and Discussion

Segregation of the structures

Thirty one different structures were considered as 'starting structures' for the high temperature MD simulations in *vacuo*. The variation in the temperature was regulated by employing the Berendsen coupling scheme.[31] All the 620 low energy structures obtained from the dynamics protocol indeed retained the L-configuration. Despite an additional input of the torsional restraint of 5 kcal/rad^2/Å^2, to minimize the *cis–trans* isomerism across the Gln-Pro peptide bond, 13 structures exhibit the existence of *cis*-isomer. The energies of 607 structures with all *trans* peptide bonds, when plotted against the frequency of occurrence, exhibited a typical bell-shaped Gaussian-like distribution (Fig. 2). According to hypothesis[19] "the structure display a Gaussian-like distribution of energies, the low-energy tail of the distribution being representative of those conformers which would be more probable in solution." Consequently, 311 low energy structures (energy ≤ -118.5 kcal/mol) were considered for the analysis.

Figure 3 shows the ϕ, ψ distribution of the 311 structures, for the Gln and Pro residues, on the Ramachandran map. Additionally, the structures that satisfied the Ramachandran criteria i.e., the backbone conformers representing all combinations of the local minima for L-residue ($-170° < \phi < -40°$, $-70° < \psi < -40°$ and $60° < \psi < 160°$; $50° < \phi < 70°$, $55° < \psi < 90°$) were considered for further analysis.[34] It should be noted that the reported ϕ, ψ distributions of 309 structures of the Gln and Pro residues are quite distinct compared to those conformations predominant in DMSO solution, as deduced from ^{1}H NMR parameters.[8] Of the 311 low energy structures, only 83 structures could satisfy the Ramachandran criteria indicated previously. While



Scheme 1.

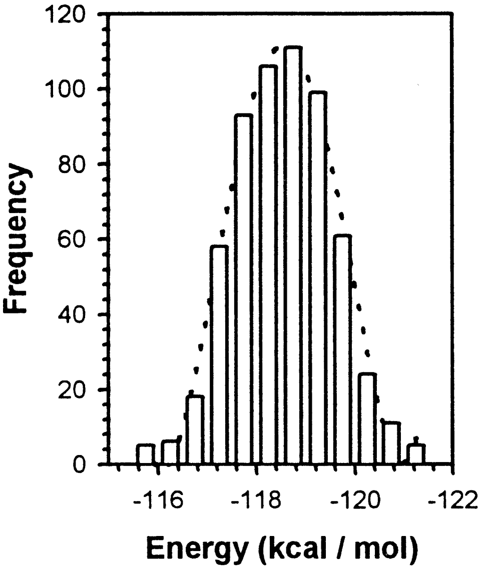


Figure 2. The Gaussian-like distribution of the energies of 607 structures, with all-*trans* peptide bonds, obtained from MD simulation studies in vacuo. The dotted line indicates the bell-shape regression.

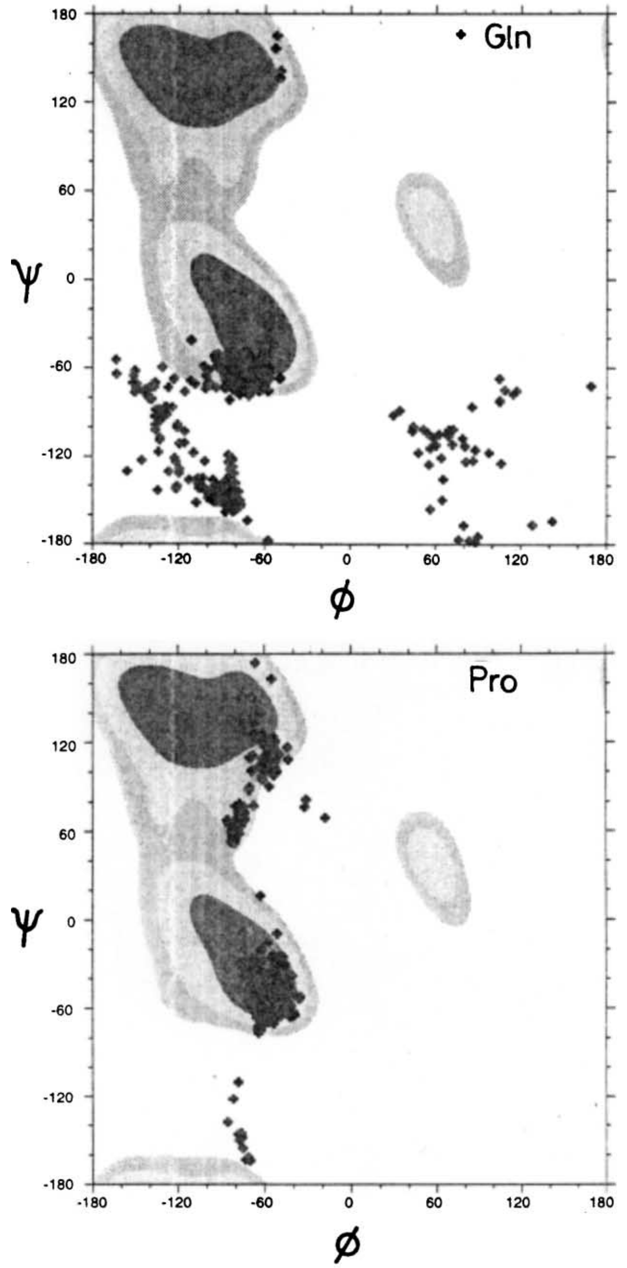


Figure 3. Ramachandran maps for the Gln (upper) and Pro (lower) residues of the 311 low energy structures of rigin.

Table 1. A comparison of the averaged torsion angles[a] (°) of the two MD simulated families: **A** and **B**, obtained for rigin in vacuo conditions; for the comparison sake the averaged torsion angles of the MD simulated structures of family **C** in *implicit* DMSO[b] are also summarized

Parameters	**A**	**B**	**C**[b]
No. of structures	64	19	34
Energy	−120.5	−119.7	−24.8
RMSD	0.43	0.41	0.56
Gly ψ_1	−151.6±4.9	−149.2±10.3	−126.5±8.3
Gln ϕ_2	66.1±2.8	−62.9±5.5	−62.0±5.4
ψ_2	66.1±2.8	−67.5±19.9	−54.9±6.8
χ_2^1	71.4±16.9	74.8±12.8	−136.1±7.9
χ_2^2	57.4±24.2	−22.0±11.4	175.0±11.3
χ_2^3	10.3±24.5	109.1±18.9	37.8±12.2
Pro ϕ_3	−65.5±2.5	−64.3±7.8	63.6±4.8
Pro ψ_3	−57.9±7.7	59.3±11.1	−46.3±10.5
Arg ϕ_4	−143.6±8.7	−115.3±21.1	−124.0±9.4
χ_4^1	−101.2±13.2	−60.9±10.5	−141.9±11.4
χ_4^2	−25.7±16.2	−177.3±11.8	175.5±21.1
χ_4^3	37.8±19.6	130.3±21.1	158.7±8.9
χ_4^4	−123.2±17.7	165.7±24.8	162.9±20.5

[a] Standard deviations are also reported.
[b] Data from ref 8.

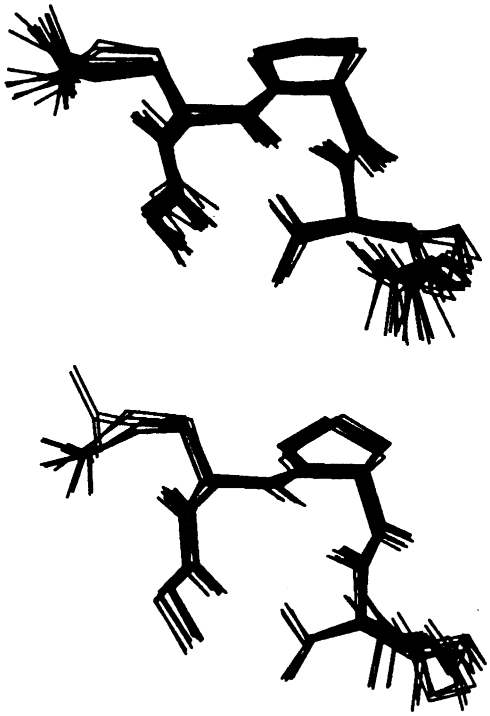


Figure 4. Superimposition of the 64 structures of family **A** (upper) and 19 structures of family **B** (lower) of rigin obtained from MD simulation studies.

considering the possibility of various definable secondary structural features (C_5-structure, γ-turns, various β-turn structures and extended conformations) that can be accessible to a short linear peptide of the size under consideration,[8] to our surprise all the 83 structures display essentially similar distributions of the backbone torsion angles and consequently, in principle, these could be grouped into one family. The RMSD value for the non-hydrogen backbone atoms, including the pyrrolidine ring of the Pro residue, was estimated to be ≤ 0.45 Å (Table 1). However, a careful analysis of these structures indicates distinct differences in the preferred side-chain conformations, as a result, the structures could readily be divided into two families **A** and **B**: depicted in Figure 4. While the 64 structures in family **A** predominantly favor a C^{γ}-*endo* conformation, the remaining 19 structures in family **B** prefer a C^{γ}-*exo* orientation.[35,36]

Proposed conformation

The averaged backbone and side-chain torsion angles of the constituent residues, summarized in Table 1, characterize the molecular conformations of rigin under the influence of distance dependent dielectric i.e., $\varepsilon = r_{ij}$. A number of interesting structural features of the energetically stable conformations of rigin are apparent. While the backbone, ϕ, ψ angles of the two families: **A** and **B**, are remarkably similar, the observed χ values of the Gln and Arg residues differ significantly. Under the condition, rigin tends to adopt a highly *folded* backbone conformation. The torsion angles across the -Gln-Pro- segment: $\phi_{i+1} \sim -65^\circ$, $\psi_{i+1} \sim -65^\circ$, $\phi_{i+2} \sim -65^\circ$, $\psi_{i+2} \sim -60^\circ$ lie in the right-handed helical-region of the Ramachandran map[34] and characterize the molecular structure as a *distorted* type III β-turn. The idealized torsion angles for a type III β-turn structure are: $\phi_{i+1} \sim -60^\circ$, $\psi_{i+1} \sim -30^\circ$, $\phi_{i+2} \sim -60^\circ$, $\psi_{i+2} \sim -30^\circ$ which often facilitate the formation of a classical 1 ← 4 intramolecular H-bond.[37–45] Interestingly, the proposed *distorted* β-turn structure is devoid of a 1 ← 4 intramolecular H-bond despite the fact that the observed distributions of $C_i \cdots C_{i+3}$ distances are significantly shorter than 7.0 Å.[40–44]

The predicted average conformations of both the families **A** and **B** exhibit the close proximity of the positively and negatively charged end groups. The averaged end-to-end distance of ~1.80 Å between the interacting atoms i.e., Gly $H_3N^+ \ldots C^{\alpha}OO^-$ Arg, strongly facilitate the fabrication of an effective '*salt-bridge*'.[44–48] The average distributions of the Gly $C^{\alpha} \ldots C^{\alpha}$ Arg distances in family **A** (4.86 Å) and **B** (4.73 Å) are typical of *tightly folded* reverse-turn structures (Fig. 5). The disfavorable orientations of the amide functionalities that is Arg N_{i+3}–H and Gly C_i=O groups, may be attributed to ψ_{Gln} and ψ_{Pro} which deviate appreciably from the idealized values of a type III β-turn structure, most probably to optimize the '*salt-bridge*'. Remarkably, our previous MD simulations in a high polarity condition ($\varepsilon = 45$) however, yielded 34 low energy structures, where the end-to-end interaction was fully-retained.[8] The retention of this feature in both polar and non-polar environments, presumably suggests that at the hydrophobic or less polar receptor site rigin tends to prefer a compact *folded* conformation. Therefore, we are inclined to propose that in MD simulated structures of rigin, irrespective of simulation conditions i.e., low versus high dielectric constants, the Arg amide-NH however, may not be locked by the C=O group.

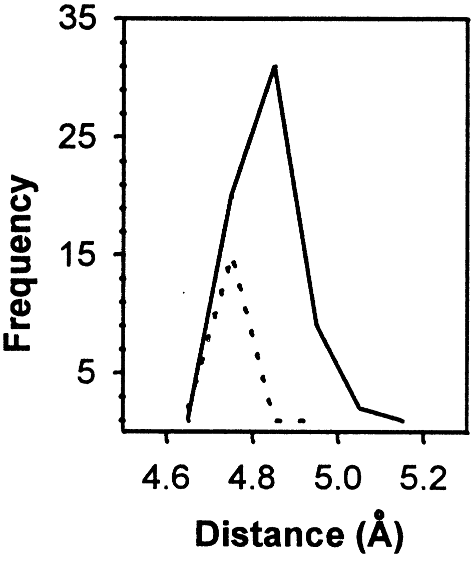


Figure 5. The distribution of interatomic distances observed between Gly $C^{\alpha} \cdots C^{\alpha}$ Arg atoms. Solid line and the dotted line represent the distribution for 64 and 19 structures for the families **A** and **B**, respectively.

The terminal Gly and Arg moieties favor an *extended* conformation, torsion angles being: $\psi_i \sim -150^\circ$ and $\phi_{i+3} \sim -130^\circ$, respectively. Attractively, the ϕ, ψ distributions of the Gln and Pro residues in the Ramachandran plots, shown in Figure 3, are strikingly similar to one of those conformers obtained in *implicit* DMSO and presented by 34 structures of the family **C** (Table 1). It should be underlined that the experimentally derived one- and two-dimensional ^{1}H NMR parameters in DMSO-d_6 could also satisfy the characteristic spatial arrangements of the residues presented by the averaged structure. Nevertheless, the preferred side-chain orientations of the Gln and Arg residues, under distinct simulation conditions, are distinctly different.

The strong preference for the ψ torsion angles of the Gln residue close to $\sim -60^\circ$ is rather interesting since, theoretical energy calculations of the terminally protected model dipeptides of the type: Ac-Xaa-Pro-$NHCH_3$ (where Xaa = Ala, Asn, Gly or Pro) have revealed that the torsion angle $|\psi| \leq 70^\circ$ of the residue preceding the proline, is disfavored due to non-bonded interactions between the C^{β} atom of the preceding residue and the C^{δ} atom of the pyrrolidine ring of the Pro residue.[38,39] The occurrence of conformationally constrained Pro residue is known to create a '*kink*' in polypeptides chains which tends to stabilize '*reverse*' turn structures.[38–42] Whereas the preference of the Pro residue at $i+1$ position (left-corner) of the β-turn structures is well documented in proteins and polypeptides, the incident of its occupying $i+2$ position (right-corner) has been largely uncommon. Of the 459 β-turn structures inspected from 29 highly refined crystal structures of globular proteins (resolution ≤2.0 Å) only 12 examples showed the occupancy of the Pro residue at the $i+2$ position.[40] Moreover, H-bonded helical conformations in globular and membrane proteins, interrupted by the insertion of Pro residues, provide compelling evidence for an accommodation of the ψ torsional angle close to $-40 \pm 20^\circ$, the experimental results that fully corroborate with the energy minimized structures.[49] Recent conformational energy calculations of Pro containing Ala-based peptides revealed highest preference of Pro at the beginning of the helical structure favored by non-boned local electrostatic interaction between the two residues preceding proline.[50] The reduced conformational flexibility of the segments preceding and following the 'Pro kink' may be critically important in restraining the overall dynamic behavior of the molecule. An accommodation of an unusual preferred folded conformation: $\psi_{Gln} \sim -60^\circ$, is suggested to be energetically compensated by a strong electrostatic interaction.

The side-chain conformations of the Gln and Arg residues of the two families **A** and **B**, exhibit distinct distributions of the torsion angles. While the family **A** with Pro C$^{\gamma}$-*endo*, prefers a highly-folded conformations for the Gln ($\chi_2^3 \approx 10°$) and Arg ($\chi_4^2 \approx -26°$; $\chi_4^3 \approx 38°$) side chains, the corresponding torsion angles of these residues in family **B** (Pro C$^{\gamma}$-*exo*) are significantly extended ($\chi_2^3 \approx 109°$; $\chi_4^2 \approx -177°$; $\chi_4^3 \approx 130°$). Remarkably, the folded *unique* disposition of the Gln side-chain clearly favors the creation of a 'main-chain to side chain' H-bond, that is $N^{\alpha}-H\cdots N^{\varepsilon}$ interaction. The averaged H-bond geometric parameters: $d(N^{\alpha}\cdots N^{\varepsilon}) =\sim 2.61$ Å and ~ 2.34 Å; $d\,(N^{\alpha}H\ldots N^{\varepsilon}) = \sim 1.80$ Å and ~ 1.73 Å and the H-bond angles: $N^{\alpha}H\cdots N^{\varepsilon} = 134.08$ and 116.97° in families **A** and **B,** respectively, establish the existence of a strong $N^{\alpha}H\cdots N^{\varepsilon}$ interaction, encompassing a *novel* seven-membered ring motif. The identification and characterization of a $N^{\alpha}H\cdots N^{\varepsilon}$ interaction is indeed stimulating since, the analysis of the Gln and Asn side-chain conformations, from high resolution protein crystal structures, unraveled that only Asn side chain has a geometry that can connect it back to the main chain.[51–57] Interestingly, the experimental proof for existence of such an energetically preferred side-chain disposition is obtained from the reported crystal molecular structure of a simple model system: Piv-N′-methyl-Gln-$NHCH_3$.[58] The finding indeed substantiate an incident of an intra-residue main-chain to side-chain H-bonding interaction across Gln residue thereby, signifying the results of present investigation. Further, despite favoring an overall highly-folded conformation of the Arg side chain, the formation of an intra-residue '*salt-bridge*' is not apparent. The viewed side-chain conformational peculiarities may indicate their specific functional importance in rigin.

The reported preliminary theoretical investigation of rigin under the influence of low dielectric constant ($\varepsilon = 3.5$), by means of energy minimizations, yielded eight low energy conformers ($\Delta U < 8$ kcal/mol).[3] The backbone torsional angles: $\psi_{Gly} \sim 155°$, $\phi_{Gln} \sim -126°$, $\psi_{Gln} \sim -121°$, $\phi_{Pro} \sim -65°$, $\psi_{Pro} \sim -45°$, and $\phi_{Arg} \sim -150°$ of the energetically most stable conformer characterized, was significantly *extended* accept across the Pro residue. On the contrary, the proposed *tightly folded* conformations, presented by family **C**, are also found to be significantly populated under the influence of higher dielectric conditions like DMSO.[8] Surprisingly, of the eight reported structures none of the conformation was appreciably folded and the authors concluded that "tuftsin-like fragment 341–344 must comprise space structure characteristic of tuftsin molecule" nevertheless, assigned relatively lesser compactness to rigin conformation was presumably due to weaker non-bonded interactions.[3] It may be noted that in both the families **A** and **B**, the observed side-chains conformations of the Gln and Arg residues are extended except the χ_3 torsion angles which prefer 'folded orientations' (Gln $\chi_3 \approx -95°$ and Arg $\chi_3 \approx 72°$). Similar structural features of the functional side chains were also viewed by Veretennikova et al.[3]

Whether in vacuo simulations correctly reproduce the hydrophobic relatively less polar membrane like environment may be argued however, over the decades inspiring predictions have been made for a number of pharmacologically important peptides and peptidomimetics and a wealth of information indispensable for '*rational drug design*' have been produced and validated experimentally.[1,7,9–12] To substantiate, the two distinct conformational models volunteered originally for tuftsin were primarily relied on theoretical energy calculations employing effective low dielectric constants. The foremost model suggested by Konopinska et al.[15] exhibited close proximity of the charged $N^{\alpha}H_3^+$ and $C^{\alpha}OO^-$ terminii whereas the conformation proposed by Nikiforovich et al.[16,17] predicted the same notable feature, instead the Lys N^{ε}-amino group was spatially close to the Arg $C^{\alpha}OO^-$group. Subsequently, Chipens et al.[59] experimentally validated the predicted models by synthesizing two tuftsin analogues i.e., a cyclic c(Thr-Lys-Pro-Arg) and a quasi-cyclic Thr-c(Lys-Pro-Arg) and evaluated their biological activity. Relied on simple molecular modeling approach, the investigators convincingly demonstrated that the latter analogue indeed represented the bio-active conformation.[59] In order to validate and advocate the approach employed for proposing the molecular model for rigin, we extended the present protocol to reassess the tuftsin conformation. Surprisingly, the overall conformational characteristics deduced for tuftsin molecule shown in Figure 6 (Ashish and R. Kishore, unpublished) exhibit remarkable similarities with those reported by Nikiforovich et al.[16] Of particular interest is the detention of the intra-molecular '*salt-bridge*' i.e., Lys $N^{\varepsilon}H_3^+\cdots C^{\alpha}OO^-$ Arg interaction. Therefore, we are inclined to propose that the ion pairing may be the principle driving force for the preferred low energy conformation of rigin and at the receptor site,

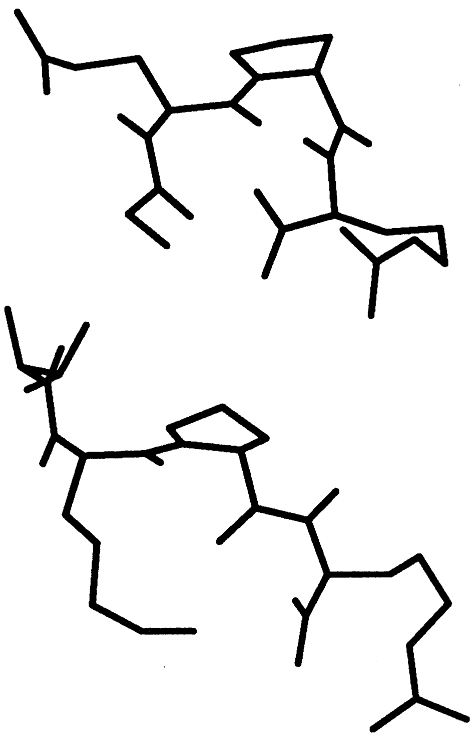


Figure 6. Predicted qualitative differences between the averaged molecular conformations of 64 structures of rigin (upper) and 28 structures of tuftsin (lower) obtained from MD simulation studies in vacuo.

the conformation may closely resemble a *distorted* type III β-turn structure. Interestingly, the relative significance of electrostatic interactions in protein folding and protein functionality have also been well recognized.[60,61] Despite the fact that half of the residues in the two equipotent immunomodulating tetrapeptides are identical they display distinct conformational features under similar simulation conditions. It may be relevant to indicate that while an unusual type VII β-turn structure predominates for rigin under the influence of a dielectric continuum representative of DMSO, a *distorted* type III β-turn structure is invariably preferred under low dielectric conditions. The critical conformational differences between the two energetically preferred i.e., a *distorted* type III β-turn and the type VII β-turn structures, accessible to rigin sequence under the simulation conditions, may lead to better understanding of the peptide–receptor complexation for the construction of non-peptidic templates leading to specific physiological response(s). The comparative conformational dissections of the two models emerged from unrestrained MD simulations, are likely to provide new insights that may be of direct biochemical relevance.

Conclusions

The results of systematic conformational searches in vacuo via MD simulations, establish that the backbone of the 83 low energy conformers of rigin show a strong preference for a tightly folded *distorted* type III β-turn structure across the central -Gln-Pro- segment. The conformational variabilities put forward in the two models **A** and **B**, under low dielectric conditions, may indicate the precise role played by subtle conformational alterations in stabilizing the bioactive conformation of rigin which may be of crucial importance for receptor binding affinity. The consequence of a folded β-turn like topology, as judged from the distributions of $C_i^\alpha \cdots C_{i+3}^\alpha$ distances, in receptor recognition have often been realized and such idiosyncratic conformational features have indeed led to the design and construction of numerous diverse pharmacophores consistent with *quasi*-cyclic, cyclic or bicyclic constrained analogues. Another interesting outcome of this investigation is the complete absence of the *conformational floppiness*, usually caused by the very low energy barriers between different structures. The inclusion of the major stabilizing interactions, i.e., a '*salt-bridge*' and a *unique* main-chain to side-chain H-bonding interaction encompassing the seven-membered ring motif across the Gln residue, may be the strategies critical for initial steps of design of relatively more rigid rigin's derivatives/peptidomimetics. Whether such interactions are precisely indispensable for the development of the analogues remains the subject of further investigation nevertheless, in the absence of X-ray structures of both receptor and the ligand, systematic MD simulations of a number of bio-active peptides, in addition to tuftsin, e.g., somatostatin, gonadotrophin-releasing hormone, bradykinin, angiotensin, etc.[7] have established the fact that in *vacuo* investigation may be a prerequisite for the exploration of bio-active conformation since, an excellent correlation with experimental results has been perceived and turned out to be important for advancing the field of structure-based drug design via computational chemistry.[62]

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