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Molecular Dynamics and Circular Dichroism Studies on Aurein 1.2 and Retro Analog

Safyeh Soufian, Hoosein Naderi-Manesh, Abdoali Alizadeh, and Mohammad Nabi Sarbolouki

***Abstract*—**Aurein 1.2 is a 13-residue amphipathic peptide with antibacterial and anticancer activity. Aurein1.2 and its retro analog were synthesized to study the activity of the peptides in relation to their structure. The antibacterial test result showed the retro-analog is inactive. The secondary structural analysis by CD spectra indicated that both of the peptides at TFE/Water adopt alpha-helical conformation. MD simulation was performed on aurein 1.2 and retro-analog in water and TFE in order to analyse the factors that are involved in the activity difference between retro and the native peptide. The simulation results are discussed and validated in the light of experimental data from the CD experiment. Both of the peptides showed a relatively similar pattern for their hydrophobicity, hydrophilicity, solvent accessible surfaces, and solvent accessible hydrophobic surfaces. However, they showed different in directions of dipole moment of peptides. Also, Our results further indicate that the reversion of the amino acid sequence affects flexibility .The data also showed that factors causing structural rigidity may decrease the activity. Consequently, our finding suggests that in the case of sequence-reversed peptide strategy, one has to pay attention to the role of amino acid sequence order in making flexibility and role of dipole moment direction in peptide activity.

***Keywords*—**Antimicrobial peptides, retro, molecular dynamic, circular dichroism.

I. Introduction

THE structure-activity relationship studies of peptides have shown that introduction of backbone modification into biologically active peptides affects potency and conformation properties, which in some cases, leads to the production of analogs with advantageous properties [1]. Retro (RE) directional isomers are modified peptides derived from native sequences. They are made up of L-amino acids in which the amino acid residues are assembled in opposite direction to the native peptide sequence[2]. So far, not a single native retro-protein has been found in nature [3].

There has been an interest in asking if retro-peptide fold at all or do they fold differently from their native-protein [4]. The significance of retro-peptide for drug design originates from properties of physicochemical and overall topological similarity between a native-peptide, and its retro sequence[5].

Literature on mimicry between native-peptide and retro peptides considers two subjects: (a) how similar or different the structures are and (b) what is functional mimicry between native and retro-peptides in biological recognition events[6]. In these studies, there are examples that often contradict each other. Similarity in activity of retro- and native peptides has been observed in a few instances [7-10]. However, there are also reports where the RE isomer has failed to mimic the original peptide [5]. Thus, there is a valid reason for more case studies in order to identify rules of predictive for the success or failure of such mimicry.

Aurein 1.2 is a 13-residue amphipathic peptide [11] with a high propensity for the α-helix formation in TFE [12]. It is one of the shortest antimicrobial and anticancer peptides that have been reported to date [13]. It is known that the effect of the peptide occurs via its interaction with the membrane's phospholipid which causes damage to the cell membrane [14].

A key point in this issue is whether aurein1.2 with an inverted sequence would fold to be a structure similar to the forward peptide and show same activity as the native sequence and if not, what is the main conformational difference between aurein1.2 and its retro-analog?

Here, we present results of molecular dynamic simulation, structural and functional studies of aurein1.2 and its retro analog.

II. Materials and Methods

A. Peptide Synthesis

Protected amino acids, resins, and all other synthesis reagents were from Bachem (Germany). All other chemicals were analytical or reagent grade (Merck, GmbH, Darmstadt, Germany). Peptides were synthesized by solid phase peptide synthesis according to Fmoc (9-fluorenylmethoxycarbonyl) methodology [*15, 16*]. The peptides were assembled manually, using a fitted glass reaction vessel contain 2-chlorotrityl chloride resin. Amino acid coupling was performed using 2(1H-Benzotriazole-1-yl)-1-1, N,N-tetramethyluronium tetrafluoroborate (TBTU), N-ethyl diisopropylamine. Fmoc deprotection was performed using 20% piperidine in dimethylformamide. Completion of coupling was carefully monitored by the Kaiser Test [17]. The final peptide was cleaved from the resin with a Trifluoroacetic acid (95%TFA) [18].

Safyeh Soufian is with Department of Biology, Arak Payame Noor University, P. O. Box 38135-1136, Arak, Iran [e-mail: s_sofian2001@yahoo.com).

Hoosein Naderi-Manesh and Abdoali Alizadeh are with Department of Biophysics, Faculty of Sciences, Tarbiat Modares University, P. O. Box 14115-175, Tehran, Iran.

Mohammad Nabi Sarbolouki is with Biophysics Dept, Inst of Biochemistry & Biophysics, University of Tehran, P. O. Box 13145-1384, Tehran, Iran.

B. Purification

The peptides were purified via high-performance liquid chromatography (HPLC) (Pharmacia, Sweden) on a Vydac C18 reverse-phase column using a water-acetonitrile gradient containing ~0.1% TFA. Then peptides lyophilized in a freeze dryer. The peptides were pure 95% as verified by HPLC on analytical Vydac C18 reverse-phase (RP) column. The purified peptides were assayed by several methods: amino acid analysis (Amino Tech, Germany) [19], HPLC [20], and electrospray mass spectrometry [21] (done at Laboratory for Biological and Medical Mass Spectrometry, Uppsala University, Uppsala, Sweden).

C. Antimicrobial Assays

The antimicrobial activity of the peptides was assessed in terms of minimum inhibitory concentration (MIC) using the standard microdilution susceptibility test[22]. The small culture of Listeria monocytogenes (ATCC1163), Leuconostoc mesenteroides (ATCC1059) and S.epidermidis (ATCC1114) were grown overnight. A fresh culture medium was inoculated with a small aliquot of the overnight culture and was incubated at 37 °C until the culture reached to its logarithmic stage (A600 ~ 0.5, 9×108 cells/ml). The culture was diluted to A600 ~ 0.001,106 cells/ml and dispensed into a 96-well plate, introducing ~105 cells per well (90 µl each). Then, 10 µl aliquots of the peptide at different concentrations (three assays for each) were added to the cultures, allowing the minimum inhibition concentration (MIC) to be measured. The plate was then further incubated overnight at 37 °C. A620 nm values were read using an Ultra Micro plate Reader [23].

D. Circular Dichroism (CD) Measurements

CD spectra were recorded on a JASCO J-715 spectropolarimeter; model J-715 (Japan) equipped with a temperature controller using quartz cells (1 mm, 10mm). The spectra were recorded at 25 °C in the ranges 190-250 nm at a scan rate of 60 nm/min. Peptide solutions were prepared by dissolving lyophilized peptide in deionized water. To investigate the influence of TFE on peptide conformation, different amounts of TFE were added in order to support formation of helical peptide . Three scans for each sample were performed and the average was calculated. The noise in the data was smoothed using the JASCOJ-715 software, including the fast Fourier-transform noise reduction routine, which allows a decrement of most noisy spectra without distorting their peak shapes. Ellipticity values were obtained in millidegrees directly from the instrument and converted to the molecular ellipticity, [θ] MRW, expressed in deg.cm2.dmol -1[24]. Peptide concentrations were measured by Waddell's approach [25].

E. Molecular Dynamic Simulation

The native structure which served as the initial structure of the native peptide is obtained from protein Data Bank (PDB). Its PDB code is 1VM5 [*12*]. The model for retro analog was built using HyperChem software [*26*] in the Alpha helix form. energy minimization rounds refined retro analog model structure (1000 steps of steepest descents followed by conjugate gradients until convergence). This 3D model was used as the starting points for molecular dynamics (MD) simulation.

Energy minimization calculations, molecular dynamics simulations and trajectory analysis were done using the Gromacs simulation package. Each of the peptides was solvated with a mixture of TFE and water and placed in a periodic truncated octahedron large enough to contain the peptide and 0.8 nm of solvent on all sides. In all cases, the temperature and the pressure were kept close to the intended values (300 K and 1 bar) by using the Berendsen algorithm [*27*]. The GROMOS96 force field [28] was used. The peptide was solvated with a mixture of TFE and spc water [29]. The LINCS algorithm [*30*] was used to constrain all bond lengths in the peptides and TFE. Electrostatic interactions were computed by particle mesh Ewald method [*31*]. Non-bonded interactions were truncated at a cut-off radius of 1.0 nm. The dielectric constant was taken as 1.0 and time step of 2 fs were used.

All atoms were given an initial velocity obtained from a Maxwellian distribution at the desired initial temperature. The first equilibration runs were followed by other runs 50-ps without position restraints on the peptide. The productions runs at constant temperature and pressure conditions, after equilibration, were 20 ns long.

All simulations and the analysis of the resulting trajectories were performed by using the GROMACS software package [*32*]. Molecular visualization was done in Swiss PDB viewer (SPDBV) environment and the secondary structure content analyses were performed with DSSP [*32*].

F. Trajectory Analysis

The radius of gyration (Rg) and root mean square deviation (RMSD) of the backbone atoms related to the structure at the end of equilibration (20 ns) were calculated. The root mean square fluctuation (RMSF) of the backbone atoms and the hydrophilic and hydrophobic Solvent Accessible Surface Areas (SASA) were also calculated. The secondary structures of peptides were analyzed by sampling trajectories every 5 ps with the DSSP program[*33*] .

III. Results

A. Antibacterial Test

The antibacterial activity of the synthetic peptides was determined as minimum inhibitory concentration (MIC) by micro-dilution susceptibility test[*34*]. For aurein1.2 and retro-analog achieved MIC values of 20 µg/ml and 300 µg/ml, respectively. This result indicates the retro isomer failed to act against microorganisms.

B. Circular Dichroism Measurements

As shown in Fig. 1, Far-UV CD spectra of the two peptides in aqueous solution at natural pH exhibit a minimum area around 200 nm, a typical characteristic of random coil proteins. Also, the negative molar elipticity between 215- 240

nm implies that a small fraction of the peptides has order structure.

In the presence of 50% TFE, on one hand, intensity of both spectra increases in this region and on the other hand, a red shift concomitant with a decrease in the intensity is observed in the negative peak at 200 nm. These results suggest both peptides have potential to acquire secondary structure under the influence of TFE. Aurein 1.2 acquires less helical content ([Θ] 222 of -15663) than the retro analog in the presence of TFE ([Θ] 222 of -17200) (Fig. 1). They also suggest retro that have a more rigid structure is more stable than the native molecule.

C. Molecular Dynamic Simulation

1. RMSD: In Fig. 2 (a), the structure's stability of both wild type and retro are shown through RMSD data. The retro-analog RMSD values are around 2.5Å-3.5Å, which shows significantly lower range than the observed values for the native molecule, which is in the range from 0.5Å to 4Å. After recording the MD trajectories, a matrix of root mean square deviation (RMSD) was constructed from the structures generated during the conformational evolution of Aurein1.2. Structures close in time and with small RMSD may belong to the same conformational family, where no substantial change in the structural occurred. The structure of the same conformational family tend to group in the shape of a square where show the existence of three structural families.
2. RMSF: The difference in RMSF between aurein1.2 and retro-analog was also clear (Fig. 2(b)), especially in the C-terminus of the peptide, indicating the importance of this terminus in activity.
3. Radius of Gyration: The evaluation of the radius of gyration Fig. 3 (a) revealed a difference between the two peptides with fluctuations between 0.54 nm-0.71 nm for the native and 0.4 nm -0.77 nm for the retro-analog. These results indicate that the retro- analog is more elongated than the native.
4. DSSP: DSSP analysis (Fig. 3(b)) reveals that both aurein1.2 and retro analog remain in an α-helical conformation throughout the simulation. It is possible to observe that despite their high flexibility, the backbone structural features of the two peptides do not change substantially throughout the dynamics. Conformations of Aurein 1.2 and retro-analog from TFE/Water mixture simulations are shown for comparison. The figures were made with the NOC program. Aurein1.2 model is a snapshot of representative conformational families that is most popular.(Fig. 3(c))
5. Minimum distance of the charge residues: We also measured the minimum distance between the charged groups as a function of time for both the native and the retro analog. Almost all of the distances were similar in both of the peptides. However, the distance between the Glu3 and Lys7 is 5Å In the native peptide. Whereas, in the retro analog, the distance between the Glu11 and Lys6 is 2 Å. (Fig. 4)
6. Dipole moment: The evaluation of the dipole moment (Fig. 5(a)) showed total dipole moment order is not the same(Fig. 5(b)), however there is larger fluctuation for the aurein1.2 (between -150 to + 120 Debyes in X-direction) than for the retro analog (-60 to +100 in X-direction) and the same is witnessed in Y and Z directions.

IV. Discussion

Antibacterial assay showed that reversed direction of aurein 1.2 significantly affects the activity of the peptide. A systematic analysis of the relationship between structural features and antimicrobial activity makes it possible to identify the features that are essential for activity.

Results of the CD spectra in TFE indicates that the reversion of the amino acid sequence for the peptide does not change its fold ability, and therefore, suggests that the order of its sequence is not critical to formation of a helix.

Our data also suggest that an inverted peptide sequence has the same amino acid composition, hydropathy profile and periodicity as the native peptide, and the periodicity of helices is maintained. This supports the finding that short helices (72 examples) keep their conformation when the sequence is inverted [36].

The MD results for peptides showed a relatively similar pattern for their hydrophobicity, Solvent-accessible surfaces, hydrophilicity, and solvent accessible hydrophobic surface. However, the result of RMSD showed native peptide exhibits a higher variation in its conformation than the RE analog, indicating conformation flexibility. The high flexibility is a distinct characteristic of aurein1.2 that has been evidenced by high-resolution 2D NMR too [*12*]. It is reasonable to note that the amino acids of the N-terminal play an important role in the stability of the peptide.[*35*] At the N-terminus, the helix geometry favours side chain to backbone hydrogen bonding so, polar residues are preferred [*35*]. In general, at N1, N2 and N3, GLU and ALA are preferred, presumably because negative side chains interact favourably with the helix dipole or NH groups while Ala has the strongest interior helix preference [*35*]. With sequence reversing , ALA and GLU are set at N2, N3 positions of the retro peptide. This leads to more stability in the retro-analog.

Result of RMSF shows more flexibility at C-terminus of the native peptide in comparison with the retro analog. These are some reasons for this observation.

First, At C-terminus unsatisfied backbone hydrogen bonds are fulfilled by interactions with backbone groups at upstream of the helix [*36*]. Doigh and Baldwin also found that Gly is the most favoured residue at the Ć-terminus position [*37*]. Thus, the decrease in the flexibility can be attributed to the position of GLY in the retro-analog, because Gly is put at Ć-position of retro peptide. Therefore, it will lose its flexibility and RMSF of C-terminus decreases. A critical aspect of designing RE analog of any peptide is the interchange of the N-and C-termini of the peptide. In case of aurein1.2 a

significant role has been attributed to the C-termini (Phe13) of the native peptides in binding to membrane [*38*].

Second, analysis of the Minimum distance of the charge residues during the simulation showed (Fig 4] possible formation of salt bridges in retro-peptide. Consequently, salt bridge between Lys6-Asp10 spaced i, i+4 have been introduced into analog of retro-peptide. Therefore, it can stabilize an alpha helix in the retro peptide.

It is well known that salt bridges formed between NH3+ and CO2- side chains, which could stabilize helix formation, acting in a similar way to disulfide bridges in a protein by constraining the side chains and therefore reducing the entropy of nonhelical states[*35*].

Thus, the retro analog appears to be significantly more rigid than the parent peptide in aqueous solution and TFE, particularly in the C-terminal, which is unstructured and mobile in the parent peptide. In summary, it seems, such flexibility in the native peptide is essential to its function [*39*].

In addition, Fig. 5 shows the dipole moment direction (x,y,z) difference and larger fluctuations for the native than for the retro analog. This implies a completely different electrostatic behavior between the native and the retro peptides, due to the direction different of charged residues. In this case seems that there are unfavourable charge-Macro dipole interactions in RE analogs.

On the other hand, electrostatic forces are long-range forces, which play a crucial role in folding of the structures and properties of biomolecules. An important contribution to these forces is due to permanent electric dipole moments. Particular arrangements of biomolecules such as the alpha-helix have large macro-dipoles, which induce strong electric fields [*40*]. More generally, the fluctuations of polar groups in proteins in response to a charge, an electric field or a conformational change, play a key role in folding the structure and binding properties [*41*]. The dipole of a polypeptide strongly depends on its conformation so it can be used as a probe of the geometry and the conformational dynamics.

When a peptide is located in an aqueous environment (with a dielectric constant, ε = 80), then hydrophobic, Van der Waals, or stacking interactions are predominant and electrostatic interactions are also possible. However, in Water/TFE or lipid membrane, electrostatic effects are enhanced up to 40-fold (according to coulomb's law) due to the low dielectric constant = 2 and dipole-dipole interactions can also become relevant. However, in aqueous solution the dipole is counteracted by an electrostatic reaction field generated by the solvent and the strength of the helix dipole may reduce drastically from its value in Water/TFE.

These explanations along with the obtained results indicate that not only flexibility, but also electrostatic interaction is an important factor in structure and activity of aurein1.2.

Overall one may say, the structure, conformation and function relationship is difficult to interpret. However as mentioned before, our findings suggest that structural similarity between aurein 1.2 and retro analog does not necessarily assure similar biological activity.

The most important structural aspect for antimicrobial activity is the order of side chains, regardless of chirality and amide bond direction. In a few cases, the reversal of amide bonds resulted in inactive hybrid analogs[5].

It has been observed that the structure of native-peptide and its retro analog are similar, but not identical. With sequence reversing, the CO-NH bonds are in opposite directions, the peptide backbones in this region assume closely similar conformations, but the side chains do not adopt the same orientation.

As we have seen, the subtle differences in structural detail can have a significant influence on peptide function. Both MD and CD method were able to detect an important difference in structure stability between retro and native that affect on activity.

V. Figures

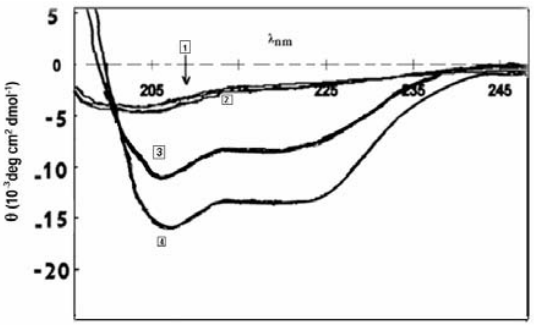

Fig. 1 Circular dichroism spectra of aurein1.2 (1)and retro-analog(2) in dionized water at 25˚C . aurein 1.2 (3) and retro-analog (4) in presence of 50% TFE

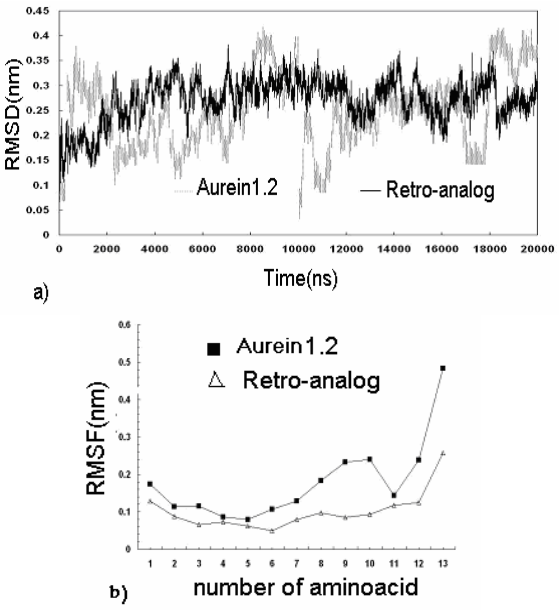

Fig. 2 (a) RMSDs versus time for the Cα atoms in the two simulations: Aurein1.2 in TFE (gray line) and Retro-analog in TFE (black line) (b) RMSF from the initial minimized structure of aurein1.2 and retro-analog in TFE

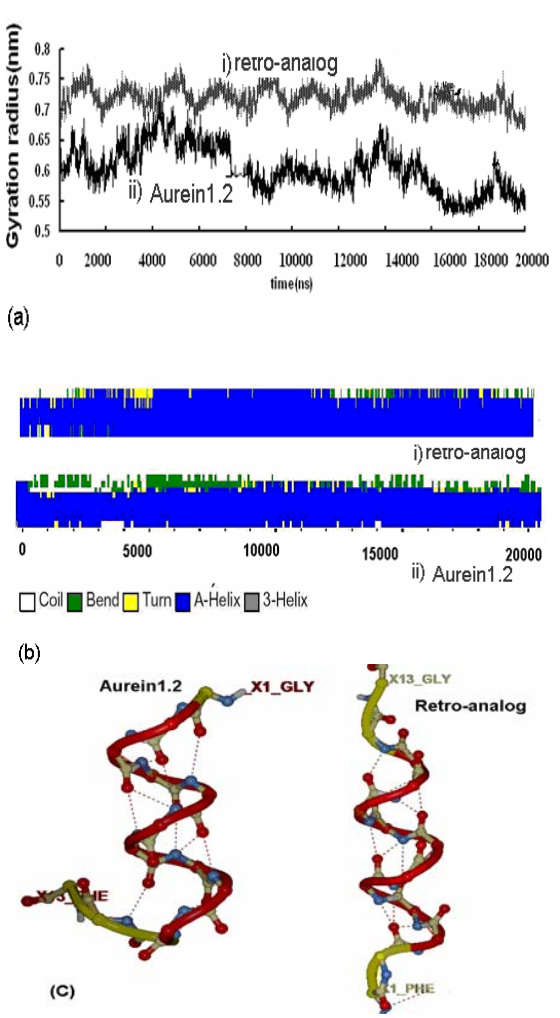

Fig. 3 Two parameters via time for molecular dynamics simulations, (a) radius of gyration as a function of time for i) Retro-analog (gray) ii) Aurein1.2 (black), (b) H-bond secondary structure, analyzed using DSSP, (c) Comparison of the conformations aurein1.2 and retro-analog. Aurein1.2 model is a snapshot of conformational family at 14000 Ps. It was made with NOC

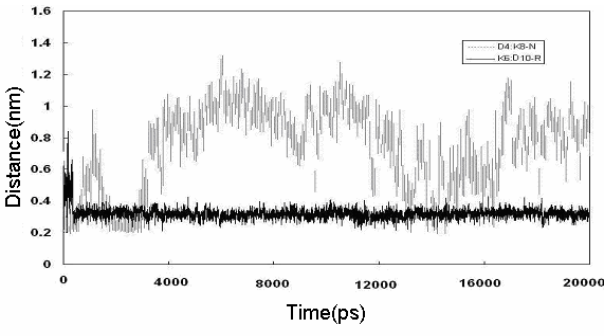

Fig. 4 Minimum distances of the of charge residues during the simulation of aurein1.2 (D4:K8, gray) and retro analog (K6:D10, black)

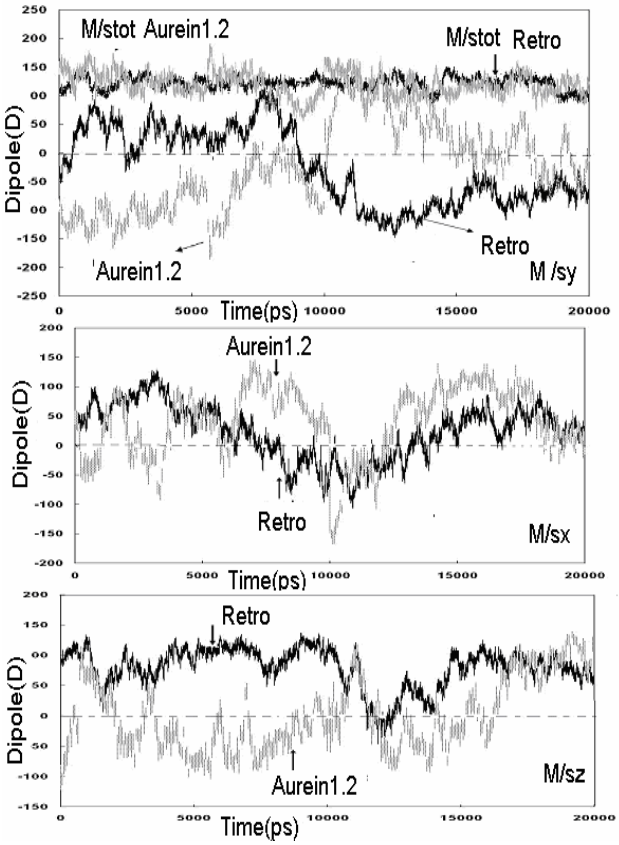

Fig. 5 Trajectory analyses of aurein 1.2(gray) and retro-analog(black) molecular dynamics simulation in TFE (dipole moment at X,Y,Z)

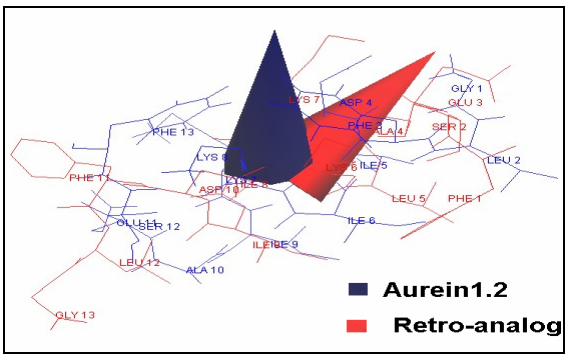

Fig. 5 (b) Total dipole moment order in Aurein1.2 and retro-analog. The figure was made with the program Chemera3

VI. Conclusion

Results of CD spectra are consistent with results from MD simulations of the retro peptide. Both indicate the peptides are as random coils in aqueous solution and adopt a particle alpha-helical conformation in TFE. The result of MD indicates the retro peptide is topochemically related to the native peptide. But there are some differences between both peptides in flexibility and dipole moment direction.

In this study, we performed molecular dynamics simulation

of aurein 1.2 and retro analog, in order to have an overview of the peptide structure, conformation and dynamic. The First observation is the parity of Results of CD spectra with the data obtained from MD simulations of the native peptide and its retro analog.

Second, both the direction and magnitude of the dipole moment in both peptides are changing. Dipole moments along the x,z,y axis directions are opposite to each other that this will lead to electrostatic behavior different between the aurein1.2 and retro analog, due to changes in sequence order. Therefore it may be said that a dipole moment direction feature is important for antibacterial activity of aurein 1.2. In addition, we observed native peptide exhibits a higher variation in its conformation than the RE analog, indicating heterogeneous conformation flexibility. Perhaps such flexibility is essential to the function of the peptide chain.

A conclusion section is not required. Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions.

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REFERENCES

[1] Leban, J. J., Kull, F. C., Jr., Landavazo, A., Stockstill, B., and McDermed, J. D. Development of Potent Gastrin-Releasing Peptide Antagonists Having a D-Pro- (CH2NH)-Phe-NH2 C Terminus. Proc. Natl. Acad. Sci 90: 1922-1926. 1993.

[2] Chorev, M. The partial retro-inverso modification: A road traveled together. Biopolymers 80 (2-3): 67-84 .2005.

[3] Peer R. E. Mittl, C. D., David Sargent, Niankun Liu, Stephan Klauser, Richard M. Thomas. The retro-GCN4 leucine zipper sequence forms a stable three-dimensional structure. PNAS 97: 2562–2566. 2000

[4] Stephan Lorenzen, C. G., Robert Preissner and Cornelius Frömmel. Inverse sequence similarity of proteins does not imply structural similarity. FEBS Letters 545: 105-109. 2003.

[5] Fischer, P. The design,synthesis and application of stereochemical and directional peptide isomers: A critical review. Current Protein & Peptide Science 4 (5): 339-356. 2003

[6] Guptasarma. FEBS Lett, Reversal of peptide backbone direction may result in the mirroring of protein structure , 310: 205–210. 1992.

[7] Zhao M, K. m. H., Mokotoff M. Briand retro-inverso peptide corresponding to the GH loop of foot-and-mouth disease virus elicits high levels of long-lasting protective neutralizing antibodies. Proc Natl Acad Sci U S 94(23): 12545-12550. 1997

[8] Meziere C, V. M., Dumortier H, Lo-Man R, Leclerc C, Guillet JG, Briand JP, Muller S. In vivo T helper cell response to retro-inverso peptidomimetics. J Immunol 159(7): 3230-3237. 1997.

[9] Carmona AK, J. L. Inhibition of angiotensin converting enzyme and potentiation of bradykinin by retro-inverso analogues of short peptides and sequences related to angiotensin I and bradykininl. Biochem Pharmaco 51(8): 1051-1060. 1996.

[10] Bonelli F, P. A., Verdini AS. . Solid phase synthesis of retro-inverso peptide analogues. Synthesis and biological activity of the partially modified retro-inverso analogue of the bradykinin potentiating peptide BPP9a (gLys6, (RS)-mPhe7, Ala8] .Int J Pept Protein Res 24(6): 553-556. 1984.

[11] Rozek, T., J. H. Bowie, J. C. Wallace, M. J. Tyler. The antibiotic and anticancer active aurein peptides from the Australian Bell Frogs Litoria aurea and Litoria raniformis. Part 2. Sequence determination using electrospray mass spectrometry. Rapid Commun. Mass Spectrom 14: 2002-2011. 2000.

[12] Rozek T., K. L. W., J. H. Bowie, I. N. Olver, J. A. Carver, J. C. Wallace, and M. J. Tyler. The antibiotic and anticancer active aurein peptides from the Australian Bell Frogs Litoria aurea and Litoria raniformis the solution structure of aurein 1.2. Eur. J. Biochem 267: 5330-5341. 2000.

[13] Dennison, S. R. W., Michelle; Harris, Frederick; Phoenix, David Anticancer α-Helical Peptides and Structure / Function Relationships Underpinning Their Interactions with Tumour Cell Membranes. Current Protein and Peptide Science 7: 487-499. 2006.

[14] Sarah R. Dennison a, F. H. b., David A. Phoenix. The interactions of aurein 1.2 with cancer cell membranes. Biophysical Chemistry 127: 78–83. 2007.

[15] Fields, G. B., Z. Tian, and G. Barany. Synthetic peptide: a user's guide. 77–183. 1992

[16] Furka, A., F. Sebestyen, M. Asgedom, and G. Dibo. General method for rapid synthesis of multicomponent peptide mixtures. Int. J. Peptide Protein Res 37: 487–493. 1991

[17] Goodman, M. Synthesis of Peptides and Peptidomimetics, Thieme/Houben-Weyl Series.2005.

[18] ChemPep Protocol,http://www.chempep.com/ChemPep-Boc-Solid-Phase-Peptide-Synthesis.htm, 2006

[19] A.J. Smith, B. J. S. AAA, Postcolumn amino acid analysis. Methods in Molecular Biology: Protein Sequencing Protocols 64,: 139–146. 1997.

[20] Vydac, G. The Handbook of analysis and purification of peptide and proteins by reversed –phase HPLC.2002.

[21] Igor A. Kaltashov, S. J. E (Ed.) Conformation and Dynamics of Biomolecules.2005.

[22] Hancock, b. in first Gordon conference on antimicrobial peptides.

[23] Hancock, R. Peptide antibiotics. Lancet 349: 418 - 422. 1997.

[24] Woody, R. Circular dichroism and conformation of unordered polypeptides. Adv biophys Chem 2: 37–79. 1992.

[25] W.J. Waddell. A simple ultraviolet spectrophotometric method for the determination of protein. J. Lab. Clin. Med 48: 311–314. 1956.

[26] HyperChem® Release 7 for Windows, Hypercube. 2002.

[27] Berendsen H. J. C., P., J. P. M., van Gunsteren, W. F., Di Nola, A. & Haak, J. R. Molecular Dynamics with Coupling to an External Bath. J. Chem. Phys 81: 3684-3690. 1984.

[28] van Gunsteren W. F., D., X. & Mark, A. E. GROMOS forcefield. In Encyclopedia of Computational Chemistry. Encycl. Comput. Chem 2: 1211-1216. 1998.

[29] van Gunsteren WF, B. S., Eising AA, Hünenberger PH,Krüger P, Mark AE, Scott WRP and Tironi IG. Biomolecular simulation: the GROMOS96 manual and user guide. 1996

[30] Hess, B., Bekker, H., Berendsen, H.J.C., and Fraaije, J.G.E.M. LINCS: A linear constraint solver for molecular simulations. J. Comp. Chem. 18: 1463–1472. 1997.

[31] Deserno, M. a. H., C. 1998. . How to mesh up Ewald sums:A theoretical and numerical comparison of various particle mesh routines. J. Chem. Phys 109: 7678–7693. 1998.

[32] Gromacs User Manual.2006.

[33] Kabsch, W. and C. Sander. Dictionary of protein secondary structure: pattern-recognition of hydrogen-bonded and geometrical features. Biopolymers 22: 2577–2637. 1983.

[34] Taylor, W. R. Identification of protein sequence homology by consensus template alignment. J. Mol. Bio 188: 233–258. 1986.

[35] Johannes Buchner, T. K. Protein Folding Handbook, Vol. 1. 2005.

[36] S Gnanakaran, H. N., John Portman. Peptide folding simulations. Current Opnion in structural Biology 13: 168-174. 2003.

[37] Doig, A. J. a. B., R.L. N- and C-capping preferences for all 20 amino acids in -helical peptides. Protein Sci. 4: 1325–1336. 1995.

[38] Ernesto E. Ambroggio, F. S., John H. Bowie, Gerardo D. Fidelio, Luis A. Bagatolli. Direct visualization of membrane leakage induced by the antibiotic peptides: Maculatin, Citropin and Aurein. Biophys J. 89: 1874-1881. 2005.

[39] H.A.Carlson. Protein flexibility is an important component of structure-based drug discovery. Curr. Pharm. Des 8: 1571–1578. 2002.

[40] R. Antoine, I. C., D. Rayane, M. Broyer, Ph. Dugourd, G. Breaux, F.C. Hagemeister, D. Pippen, R.R. Hudgins and M.F. Jarrold,. Electric dipole moments and conformations of isolated peptides. Eur. Phy. J. 20: 583. 2002.
[41] Simonson. Dielectric relaxation in proteins: macroscopic and microscopic models. Int J Quantum Chem 73: 45-57. 1999.
[42] Persson, S., Killian, A., and Lindblom, G. Molecular ordering, and 2H-NMR, Biophys. J. 75: 1365–1371. 1998.