MOLECULAR DYNAMICS OF NEUROMEDIN NmU-8 NEUROPEPTIDE

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Molecular dynamics simulations were performed for neuromedin NmU-8, the regulatory peptide isolated from porcine spinal cord. A single NmU-8 molecule was modeled in vacuum as well as in water. In the latter case it was surrounded by 264 SPC water molecules and a periodic boundary condition was applied. A large flexibility of the Tyr^1-Phe^4 amino acids sequence was observed in vacuum in contrast to water simulation. The Arg^5- Asn^8 backbone can adopt only a limited number of conformations, while the side chains may populate all three major rotamers.

Introduction

Neuromedin U-8 (NmU-8) is regulatory peptide initially isolated in 1985 from porcine spinal cord and then found in variety of mammals, birds, and reptile [1-5]. Besides its roles in smooth muscle contraction (human ileum, urinary bladder, rat stomach etc) NmU-8 has also been implicated in hypertension, blood flow in intestine, and neurotransmission. NmU-8 is present in nerves throughout the Gl-tracts, corticotrophs within the anterior and lobe of rat and human pituitary glands, parafollicular cells in rat thyroid gland, and in various regions of brain (spinal cord, hypothalamus, substantia nigra, hippocampus, amygdala). Low levels of NmU-8 are also found in human adipose tissue lymphocytes, and spleen. NmU-8 has a primary structure as follows: Tyr^1-Phe^2-Leu^3-Phe^4-Arg^5-Pro^6-Arg^7-Asn^8. In our previous paper, the theoretical conformational analysis method was employed to study the global conformational structure of the NmU-8 octapeptide [6]. Twelve types of stable conformations with significantly different values of dihedral angles are possible. The following study [7] was shown that substitution of the L-amino acid with corresponding D-amino acid at the various position leads to the formation of two possible binding sites to neuromedin NmU-8 receptors. One of them, the N-terminal fragment Tyr^1-Phe^4 has an extended backbone form, another C- terminus capable forms β-turn on the Arg^5-Asn^7 tetrapeptide fragment. A molecular conformation is largely determined by its environment, so the aim of this present work is the study the differences in the conformation of the NmU-8 neuropeptide in a vacuum and in aqueous environment using a molecular dynamics method.

Computational method

Molecular dynamics (MD) simulations were performed for NmU-8 neuropeptide in vacuum as well as in water solution using modeling package [8]. MD is widely applied to the study of biological systems, providing insight into the structure, function, and dynamics of biological molecules [9,10]. A wide range systems have been treated, from small molecules to proteins, in vacuum and in the presence of solvent [11, 12]. Molecular dynamics simulations generate trajectories of atomic positions and velocities and some general thermodynamic properties. MD involves the calculation of solutions to Newton's equations of motions. Often an MD trajectory will become trapped in a local minimum and will not be able to step over high energy conformational barriers. Thus, the quality of the results from a standard MD simulation is extremely dependent on the starting conformation of the molecule. So, the twelve structures, including the best and the worst of the calculated structures from [6] were used as starting conformations for molecular dynamics simulations φ, ψ and χ_1 angles were analyzed for changes in conformation. Runs were performed for 300ps at 300K. The total length of the simulation depend on the system being studied and the type of information to be extracted. For example, in simulations of biological system a time step of 1 femtosecond is commonly used. To ensure that information about the highest frequency in the system is retained, generally the bond stretching frequency of water, the trajectory has to be recorded at an interval no larger than 4 femtoseconds. The length of the simulation (after equilibration) has to be long enough to enable the slowest modes of motion to occur. The force field parameters were those of the all atom version of AMBER by Cornell et al [13]. The total number of the water molecules was 264. A harmonic force towards the center of the sphere was added to atoms when they moved out of the sphere. The nonbonded cutoff distance was 12Å. The time step was 0,5fs. The program Hyper. Chem. 7.01 [14] was used for the MD simulations. All of the simulations were carried out for $2\cdot10^6$ to $1\cdot10^7$ steps.

Results and discussion

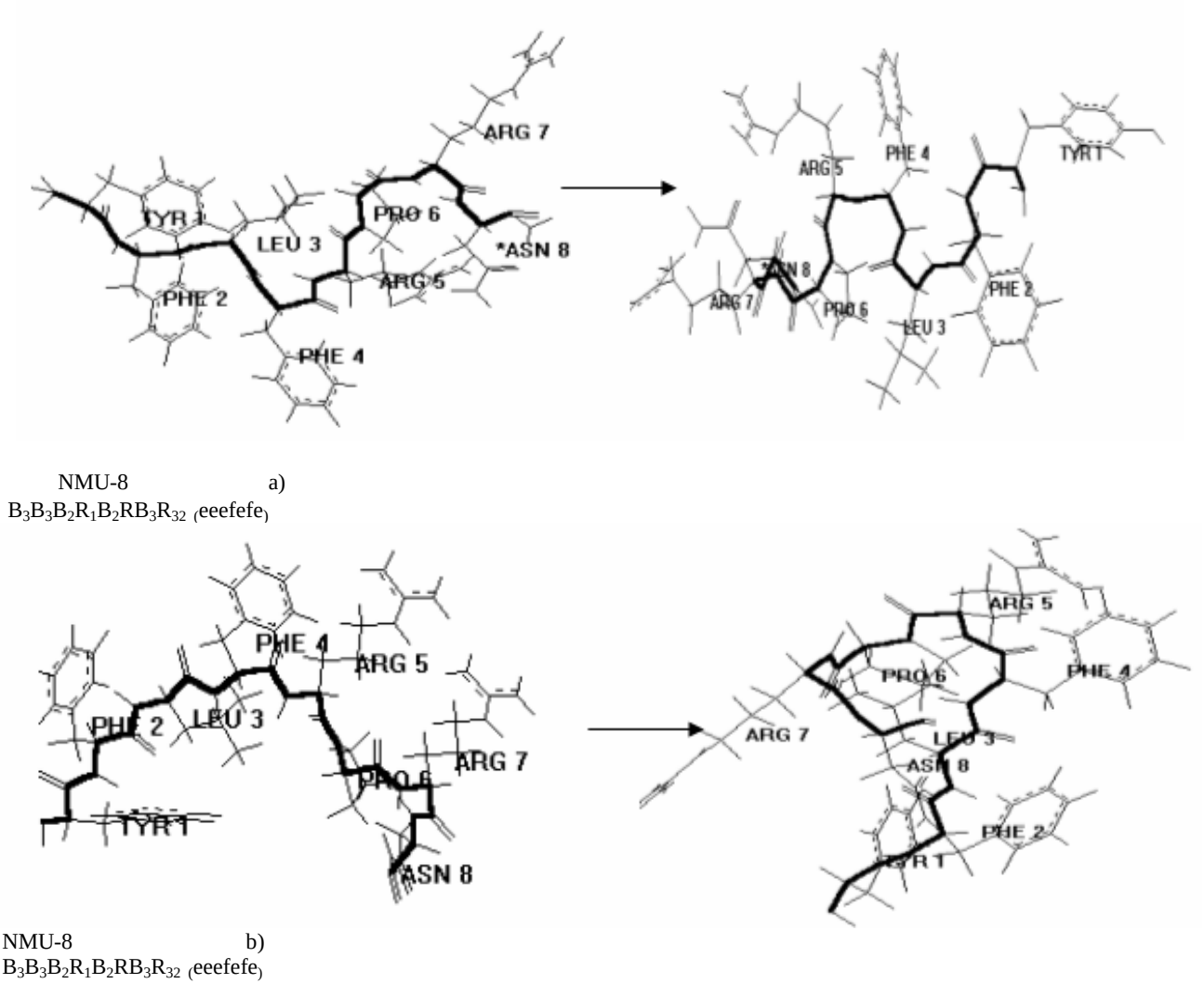
MD runs, using the 12 starting structures of NmU-8 from [6] were shown the significant differences in the conformations of the molecule in a vacuum and in an aqueous environment. Structures from the last 15ps of the run were energy-minimized and two of these are shown in Fig1. Corresponding changes of the potential energy and dihedral angle values are presented in Table 1. MD simulations show that the NmU-8 molecule backbone can adopt only a limited number conformations while the sidechains of the residues may populate all three major rotamers. A large flexibility of the Tyr^1-Phe^4 amino acids sequence was observed in vacuum in contrast to water simulation. The Arg^5-Asn^7 tetrapeptide fragment was found to be rigid in the conditions studies. Changes in intramolecular energy during simulations in water were negligible; they did not exceed 10-15 kJ/mol for NmU-8 molecule. At the same time, the molecule interaction energy was much higher due to the flexibility of the Tyr^1-Phe^4 part of the NmU-8. Interactions between aromatic side chains of the Tyr^1, Phe^2 and Phe^4 amino acids make the largest contributions to the global energy of the simulated molecule. Undoubtedly this contribution is overestimated in the vacuum approximation. The proline residue at position 6 makes an angle to the plane of the cycle and is seen to fluctuate by

±30° about a mean position during the molecular dynamics simulations. The backbone structure comprises a type II β-turn formed by residues Arg^5- Pro^6- Arg^7- Asn^8 contains a hydrogen bond between Arg^5 CO and Asn^8 N$^{\delta}$H. Now consider the conformational properties of each amino acid residue in detail. The molecular dynamics simulations revealed the possible deviation by ±120° from the optimal values of φ angle for Tyr^1 in vacuum as compared to ±20° in water. The deviations of ψ for Tyr^1 by ±80° from its optimal values are allowed in all calculated structures in vacuum and water environment. The low energy changes of χ_1 and χ_2 for Tyr^1 from 166 to 182° and from 70 to 89°, respectively, are possible. As can be seen from Table 1 of the Phe^2 and Phe^4 side chains are close to the minima of the torsional potential χ_1=60,180° and χ_2=90°. The deviations by ±20° from minimal values are possible for χ_1 angle. The rotation of the χ_2 angle for Phe^2 and Phe^4 is considerably limited due to the effective interactions between the Phe^2 and Phe^4 amino acids.

The mobility of the backbone and side chain of the Leu^3 is more restricted as compared to preceding residues of NmU-8 in vacuum as well as in water. In contrast to water simulations, where the φ angle for Leu^3 may be changed by ±20° from its optimal value, it is very mobile in vacuo. The possible deviations of ψ angle for Leu^3 in the later case are ±40°. Calculated results indicate that χ_1-χ_4 angles for Leu^3 have a noticeable conformational flexibility. All side chains angles (Leu^3) were seen to be well-defined around 180° throughout the runs.

As can be seen from Table1. the mobility of the Arg^5-Asn^8 amino acids stretch is considerably limited. So, the flexibility of φ and ψ angles of residues in the 5th and 7th positions is limited by 10° as compared to the preceding part of NmU-8. This fact can be explained due to the important role of these residues in the formation of β-turn.

Each φ angle varied about a single value, close to one of the set of possible angles calculated from molecular mechanics energy minimization [6]. The run with the high energy starting structure had an initial φ angle for Arg^5 around -60° which, during the run flipped to a value around -120°. The χ_1 angles of Arg^5 and Arg^7 were seen to vary between all 3 rotamers (-60, 60 and 180°) in the 5 runs while that for Asn^8 (χ_1) had only values around -60° and 60°.



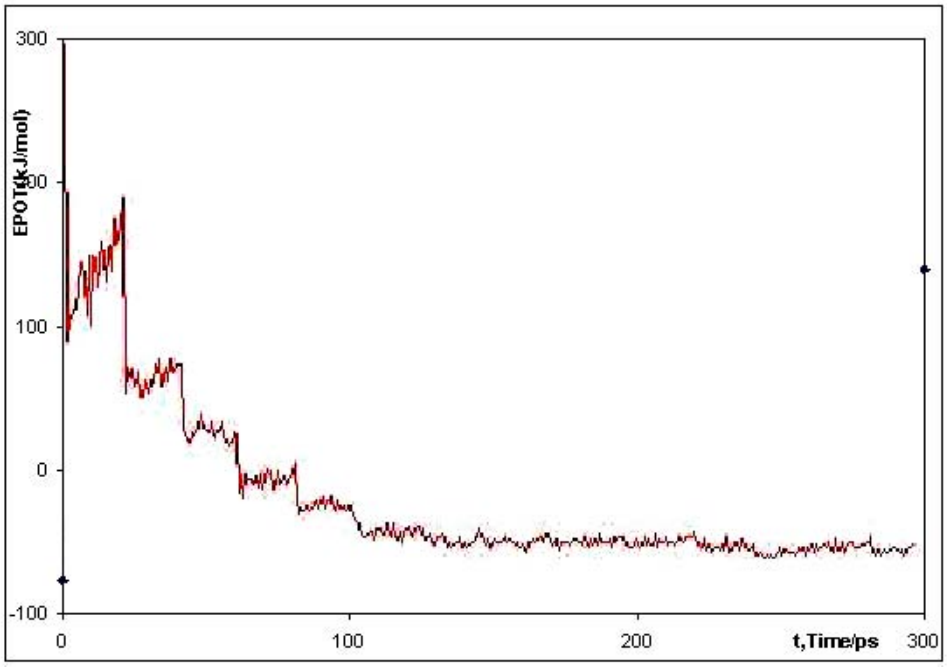
NMU-8 a)
$B_3B_3B_2R_1B_2RB_3R_{32}$ (eeefefe)

NMU-8 b)
$B_3B_3B_2R_1B_2RB_3R_{32}$ (eeefefe)

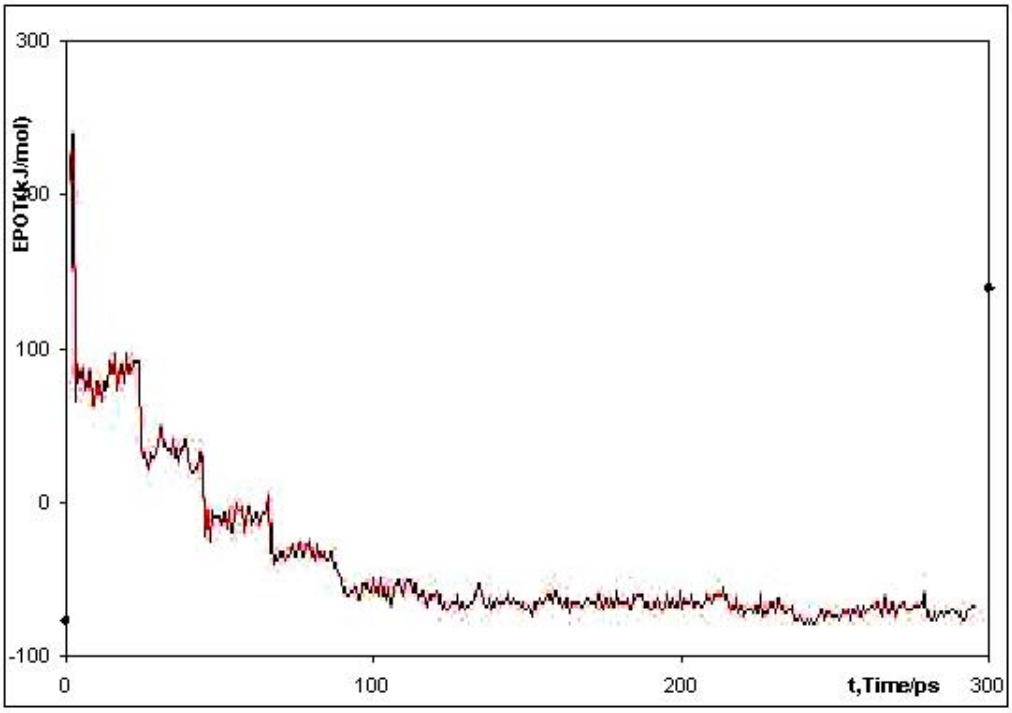
Fig.1 Computer-optimized structures of the NmU-8 neuropeptide in vacuum (a) and water (b). The initial conformation was an extended structure [6].

The potential energy changes during the MD simulations were examined (Fig 2). As can be seen, during the global sampling stage the potential energy rapidly decreased from 2000 to 337 kJ/mol. During the MD the energy changed

frequently and sometimes even experienced dramatic changes, suggesting the sampling of a fairly conformational space.The lowest energy obtained was –84 kJ/mol and corresponded to state of NmU-8 as shown in Fig.1 (b).



a)



b)

Fig.2. Energy of the NmU-8 neuropeptide during an MD simulation: a) in vacuum b) in water

Conclusion

We have carried out detailed analysis of the flexibility of the NmU-8 neuropeptide molecule by employing the molecular dynamics method. The foregoing results and discussion lead to the following conclusions:

(I). molecular dynamics simulations in vacuum as well as in aqueous solution confirm the considerable flexibility of the Tyr^{1} -Phe^{4}- sequence of NmU-8 neuropeptide;

(II). the turn conformations on Arg^{5}- Asn^{8} seqment of NmU-8 similar to the type II β-turn were more stabilized in water, with the predominant hydrogen bond between Arg^{5} NH and Arg^{7} C'O than the extended conformations.

The determined structure may be used as the basis for the design of further peptidic and/or non- peptidic selective antagonists.

Table 1.

The permissible ranges of values (in degrees) of φ, ψ, χ_1-χ_4 dihedral angles of NmU-8 neuropeptide under MD simulations in vacuum (upperline) and water (under line)

Amino acid residue	φ	ψ	χ_1	χ_2	χ_3	χ_4
Tyr^1	-63 to 160 43 to 83	54 to 210 120 to 140	166 to182	70to89	181	-
Phe^2	-25 to -110 -90 to -105	120 to 140 140 to 160	150 to176 160 to182	90(±5)	-	-
Leu^3	-60 to -117 -90 to -105	80 to 110 97 to 105	195	171	192	180
Phe^4	-83 to -105 -90 to -107	-23 to -60 -30 to -45	60(±5)	89(±2)	-	-
Arg^5	-110 to -129 -125 to -129	144 to 157 150 to 150	192	180	179	180
Pro^6	-	-82 to -90 -87 to -92	-	-	-	-
Arg^7	-120 to -125 -122 to -125	101 to 110 101 to 110	-60	180	180	180
Asn^8	-90 to -92 -90 to -92	-60 to -66 -60 to -66	-52	-80	180	-

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NEYROMEDİN NmU-8-İN MOLEKULYAR DİNAMİKASI

Donuzun onurğa sümüyündən alınmış tənzimləyici peptid neyromedin NmU-8-in molekulyar dinamika üsulu ilə konformasiya mütəhərrəkliyi öyrənilmişdir. NmU-8 üçün hesablamalar molekulun ölçüləri verilməklə vakuumda və su mühitində aparılmışdır. II-ji halda peptid 264 SPC-su molekulundan ibarət düzbucaqlı qutuya salırlar. Müəyyən edilmişdir, ki N-uclu Tyr^1- Phe^4 fraqmentinin mütəhərrəkliyi vakuumda və su mühitində qeyri-müəyyən quruluşa malikdir. Əsas zəncirin Arg^5- Asn^8 fraqmenti məhdud sayda konformasiya halında olmasına baxmayaraq, yan zəncirlər bütün mümkün hallarda ola bilirlər.

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МОЛЕКУЛЯРНАЯ ДИНАМИКА НЕЙРОМЕДИНА NmU-8

Методом молекулярной динамики изучена конформационная подвижность нейромедина NmU-8, регуляторного пептида, выделенного из спинного мозга свиньи. Расчет молекулярной динамики NmU-8 проводился в вакууме и в условиях явно заданных молекул воды. В последнем случае пептид помещали в прямоугольный ящик с 264 молекулами SPC- воды с наложенными периодическими граничными условиями. Установлено, что благодаря подвижности N- концевого фрагмента Tyr^1- Phe^4 пептид имеет неупорядоченную структуру в вакууме и в водном растворе, а фрагмент Arg^5- Asn^8 основной цепи принимает ограниченное число конформационных состояний.

Received: 22.07.03