

MOLECULAR DYNAMICS STUDIES OF THE TACHYKININ NEUROPEPTIDES

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ABSTRACT

The conformational flexibilities of two tachykinin neuropeptides, substance P and physalaemin, have been studied by molecular dynamics method in the different conditions. At the first stage the conformational changes of these peptides were studied in vacuum, but in the second stage they were surrounded by water molecules with the periodic boundary conditions. Both molecules were observed in vacuum and in water with large flexibility of the N-terminal parts of its amino acid sequences. It is shown that C-terminal backbone parts of these molecules save an alpha-helical conformation, but side chains of residues may exist in more than one orientations in all conditions.

Key words: substance P, physalaemin, conformation, molecular dynamics simulation.

TAXİKİNİN NEUROPEPTİDLƏRİN MOLEKULAR DİNAMİKASI

XÜLASƏ

Substansiya P və fisalaemin taxikinin neuropeptidlərin müxtəlif mühitlərdə konformasiya çevikliyi molekulyar dinamika üsulu ilə öyrənilmişdir. Bunun üçün peptidlərin konformasiya dəyişmələri əvvəlcə vacuumda müəyyən olunmuşdur. Sonrakı modelləşdirmə mərhələsində isə peptidlər su molekullarından ibarət qutuya salınmışdılar. Hesablamalar nəticəsində göstərilmişdir ki, hər iki molekulların N-uclu hissələrinin əsas və yan zəncirlərinin çevikliyi C-uclu hissəyə nisbətən vacuumda və su mühitində daha çoxdur. Peptidlərin C-uclu hissəsinin əsas zəncirinin alfa spiral quruluşu hər iki mühitdə daha dayanıqlıdır.

Açar sözlər:

Introduction

Tachykinins are a family of biologically active peptides distributed in the central and peripheral nervous system. The earliest known members of the tachykinin family are those that are present in mammalian systems. Tachykinins elicit a wide and complex array of biological responses, such as the stimulation of extravascular smooth muscle, powerful vasodilation, hypertensive action, activation of immune system, regulation of pain transmission, and neurogenic inflammation. The tachykinin peptides are characterized by a common C-terminal

sequence, Phe-X-Gly-Leu-Met-NH₂, where X represents either an aromatic (Phe, Tyr) or a branched aliphatic (Val, Ile) amino acid [1-3]. The C-terminal region or the message domain is considered to be responsible for activating the receptor. The divergent N-terminal region or the address domain varies in amino acid sequence and length and is postulated to play a role in determining the receptor subtype specificity. Three pharmacologically distinct receptor subtypes have been identified and cloned for tachykinins designated as NK-1, NK-2, and NK-3, which all share a significant sequence similarity. All tachykinins bind to

all the receptor subtypes, with substance P and physalaemin preferring NK-1 [4,5]. The wide range of physiological activity of tachykinins has been attributed to the lack of specificity of tachykinins for a particular receptor type. This lack of specificity can be accounted for by the conformational flexibility of these short, linear peptides. The characterization of the biologically active conformation, which controls receptor binding and subtype selectivity, is of significant interest. The most studied tachykinin, substance P (SP), was first isolated from equine brain and intestine. Its amino acid sequence was identified as Arg1-Pro2-Lys3-Pro4-Gln5-Gln6-Phe7-Phe8-Gly9-Leu10-Met11NH₂. SP is involved in transmission of pain, causes rapid contractions of the gastrointestinal smooth muscle, and modulates inflammatory and immune responses [1-6]. In spite of extensive structural research of the SP and its analogs by different spectroscopic methods, it is very difficult to determine selective antagonists of tachykinin receptors. Physalaemin is a undecapeptide with the sequence pGlu1-Ala2-Asp3-Pro4-Asn5-Lys6-Phe7-Tyr8-Gly9-Leu10-Met11NH₂. It was first isolated from the *Physalaemin fuscumaculatus* amphibian skin [7,8]. It is known that SP and physalaemin interact with one subtype of NK receptors, therefore physalaemin in the literature is often called as a structural analog of substance P [9]. For understanding of how ligand interact with their receptor is required the knowledge of the conformational specificity and dynamics of the native molecule allowing a rational design of compounds acting selectively at the tachykinin receptor level. The major aim of the present article is the investigation of the conformational dynamics for substance P and physalaemin, with the purpose of getting insight into basic structural requirements that determine ligand-receptor interaction. At the present article the confor-

mational dynamics of substance P and physalaemin have been investigated by molecular dynamics method in vacuum and in water molecules surrounding. In general, it has been found that the tachykinins display some elements of secondary structure in appropriate solution environment, though it has been suggested that they do undergo rapid conformational exchange. There are no discernible trends in the conformation of the address segments of these peptides. However, the message domains are similar in each case. In general, the message domain of these peptides undergoes conformational averaging in aqueous environments. In hydrophobic environments, the message domain assumes helical conformations or exists as a series of turns in dynamic equilibrium. It has been postulated that the binding of neuropeptides to their cell surface receptors may be catalyzed by non-specific interactions with membrane lipids and the binding of the peptide to the receptor occurs in at least two sequential steps: the binding of the peptide to the membrane, followed by the binding of the peptide to the receptor in the membrane. Although the neuropeptides in aqueous solution exist as randomly distributed conformers, the biologically active forms of these neuropeptides are likely to be ordered and stabilized within the lipid bilayers of the cell membrane before binding with their receptors. In our previous papers, the theoretical conformational analysis method was employed to study the spatial structures of the SP [10,11] and physalaemin [12] molecules. Some types of stable conformations with significantly different values of dihedral angles are determined for both tachykinins. A molecular conformation is largely determined by its environment, so the aim of this present work is the study the differences in the conformations of the tachykinin

peptides in a vacuum and in aqueous environment using a molecular dynamics method.

Computational method

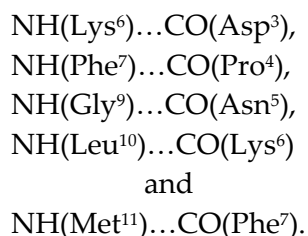
To understand the physical and structural properties of membrane-bound peptides and proteins and their relationship to the biological activities, Molecular dynamics (MD) simulations with everimproving force fields and longer time scales have been providing molecular level details of such systems. MD simulations were performed for neuropeptides in vacuum as well as in water solution using modeling package [13]. MD is widely applied to the study of biological systems, providing insight into the structure, function, and dynamics of biological molecules [14,15]. A wide range systems have been treated, from small molecules to proteins, in vacuum and in the presence of solvent [16,17]. 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 low-energy structures, including the best and the worst of the calculated structures from [11] and [12] were used as starting conformations for molecular dynamics simulations ϕ , ψ and χ angles were analyzed for changes in each conformation. Runs were performed for 300 ps 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 fem to second is

commonly used. To ensure that information about the highest frequency in the system is $\pm 30^\circ$ about a mean position during the molecular dynamics simulations. 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 [18]. A harmonic force towards the center of the sphere was added to atoms when they moved out of the sphere. The non-bonded cutoff distance was 12Å. The time step was 0,5fs. The program Hyper. Chem. 7.01 [19] was used for the MD simulations.

Results and discussion

Preliminary theoretical conformational analysis of SP has shown that its spatial structure may be described by five families of low-energy conformations with identical structure of the C-terminal heptapeptide. The C-terminal part (residues 5-11) of the SP can adopt a partially helical structures, but the N-terminal part is different in each family. Only four low-energy conformations of the SP are fall in the 0-5 kcal/mol energy interval. It is shown that two preferred conformations have very similar backbone form and values of the relative energy. In these conformations only Gln⁵ residue is in the different backbone forms. Both conformations contain some turn at the N-terminal tripeptide, but the first conformation have β -turn structure at the Pro⁴-Gln⁵-Gln⁶-Phe⁷ segment also. These β -turns are confirmed by distance between C ^{α} atoms of the *i* and *i*+3 residues (< 7 Å). The lowest energy structures of SP exhibit the most favourable dispersion contacts and therefore may be expected to become the most preferred in a strongly polar medium, when electrostatic interactions do not play a significant role. The lowest energy α -helical structure at the C-terminal

fragment is stabilized by network of hydrogen bonds: NH(Phe⁸)-CO(Gln⁵), NH(Gly⁹)-CO(Gln⁶) and NH(Met¹¹)-CO(Phe⁷). Theoretical conformational analysis of physalaemin have been indicated four families of low-energy conformations with similar C-terminal heptapeptides. Unlike the molecule of substance P, this analysis has shown that physalaemin can form one global, i.e. the lowest-energy structure, which is consist one β -turn on Asp³-Pro⁴-Asn⁵-Lys⁶ segment and on the C-terminal part α -helical segment, formed follow hydrogen bonds:



MD simulation for Substance P.

MD simulation, using the four starting lowest energy structures of each molecule from [6] and [7] were shown the significant differences in the conformations of the molecule in a vacuum and in an aqueous environment. Structural reorganization of the global conformation of substance P at the molecular dynamic simulation in vacuum and in water solution are shown in Fig1. Figure 2 shows the global structure of the substance P as a result of the molecular simulation of aqueous environment. The MD simulations revealed the possible deviation by $\pm 10^\circ$ from the optimal values of ϕ, ψ, ω, χ dihedral angles in vacuum as compared to $\pm 20^\circ$ in water. The permissible changes of values (in degrees) of ϕ, ψ, ω, χ dihedral angles of substance P lowest energy conformation under MD simulations in vacuum and water are represented in the Table 1. The deviations of ψ for Arg1 by $\pm 20^\circ$ from its optimal values are allowed in all calculated structures in vacuum and

water environment. The low energy changes of χ_1 for Arg1 from 182 to 88° are possible. As can be seen from Table 1 of the Phe7 and Phe8 side chains are close to the minima of the torsional potential. The deviations by $\pm 20^\circ$ from minimal values are possible for χ_1 angle. The rotation of the χ_2 angle for Phe7 and Phe8 is considerably limited due to the effective interactions between the Phe7 and Phe8 amino acids. The mobility of the backbone and side chain of the Leu3 is more restricted as compared to preceding residues of molecules in vacuum as well as in water. In contrast to water simulations, where the ϕ angle for Lys3 may be changed by retained, generally the bond stretching frequency of water, the trajectory has to be recorded at an interval no larger than 4 femtoseconds. Corresponding changes of dihedral angle values are presented in Table 1. MD simulations show that the molecule backbone can adopt only a limited number conformations while the side chains of the residues may populate different rotamers. A large flexibility of the Arg1-Pro4 amino acids sequence was observed in vacuum in contrast to water simulation. The Gln5-Met11 heptapeptide 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 molecule. At the same time, the molecule interaction energy was much higher due to the flexibility of the Lys3-Gln6 part of the peptides. Interactions between aromatic side chains of the Phe7 and Phe8 amino acids make the largest contributions to the global energy of the simulated molecule. Undoubtedly this contribution is overestimated in the vacuum approximation. The Pro4 fluctuates by $\pm 20^\circ$ from its optimal value as shown in Table1, i.e. Pro4 is very flexible in vacuum.

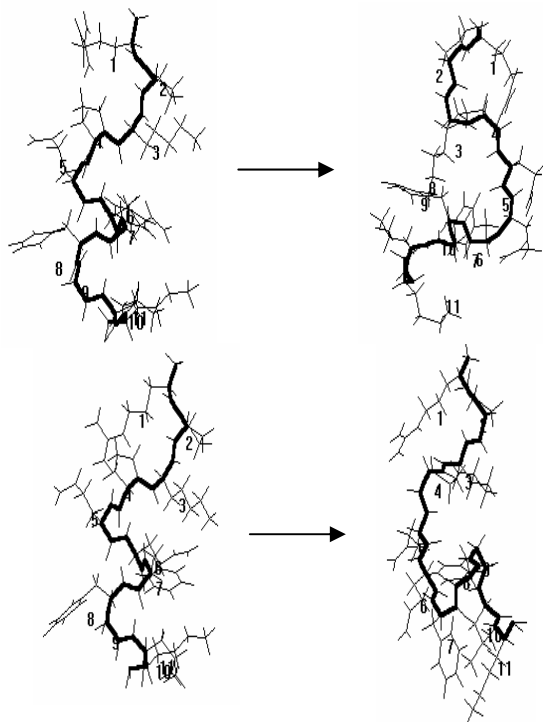


Fig.1. Conformational reorganization of the SP molecule at the molecular dynamic simulation in vacuum (at the top) and in water (below). The initial conformation take from [11].

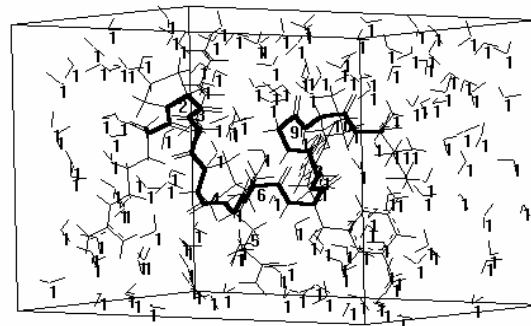


Fig.2. The final structure, received by means of MD simulation, after computer-optimization of the lowest-energy conformation of SP molecule in box with water molecules.

Table 1. The permissible changes of values (in degrees) of $\varphi, \psi, \omega, \chi$ dihedral angles of substance P lowest energy conformation under MD simulations in vacuum (upperline) and water (under line)

Residue	Backbone angles			Side chain angles				
	φ	ψ	ω	χ_1	χ_2	χ_3	χ_4	χ_5
Arg ¹	-120 to -120	117 to 111	187 to 193	184 to 168	175 (+2)	181	178	
	-120 to -120	115 to 115	185 to 183	182 to 88	173 (+2)	184	178	
Pro ²	-	-51 to -70	182 to 183					
		-51 to -51	182 to 182					
Lys ³	-119 to -134	99 to 94	180 to 171	-60 to -61	181 (-2)	180	180	180
	-120 to -120	100 to 102	179 to 182	-60 to 60	180 (-2)	180	180	180
Pro ⁴	-	-57 to -78	181 to 186					
		-42 to -52	173 to 175					
Gln ⁵	-118 to -63	158 to -37	179 to 187	-59 to 64	180 (+2)	90		
	-145 to -151	158 to 160	181 to 178	180 to 70	180 (+2)	91		
Gln ⁶	-72 to -74	-34 to -38	180 to 178	181 to 183	179 (+2)	90		
	-95 to -93	132 to 150	175 to 179	182 to 179	178 (-2)	92		
Phe ⁷	-65 to -62	-46 to -42	180 to 184	179 to 177	90			
	-106 to -108	145 to 153	180 to 182	-57 to -58	90			
Phe ⁸	-79 to -77	-32 to -33	-184 to -186	175 to 176	90			
	-122 to -114	-46 to -45	-187 to -186	-57 to -61	89			
Gly ⁹	-63 to -61	-39 to -38	-178 to -180					
	-61 to -62	-36 to -37	-179 to -178					
Leu ¹⁰	-82 to -81	-62 to -64	-174 to -171	176 to 175	64	60	57	
	-88 to -88	-63 to -65	-174 to -172	175 to 177	64	60	57	
Met ¹¹	-93 to -91	-54 to -52	181 to -181	-60 to -59	180	180	180	
	-93 to -94	-53 to -54	-181 to -180	-59 to -56	180	181	180	

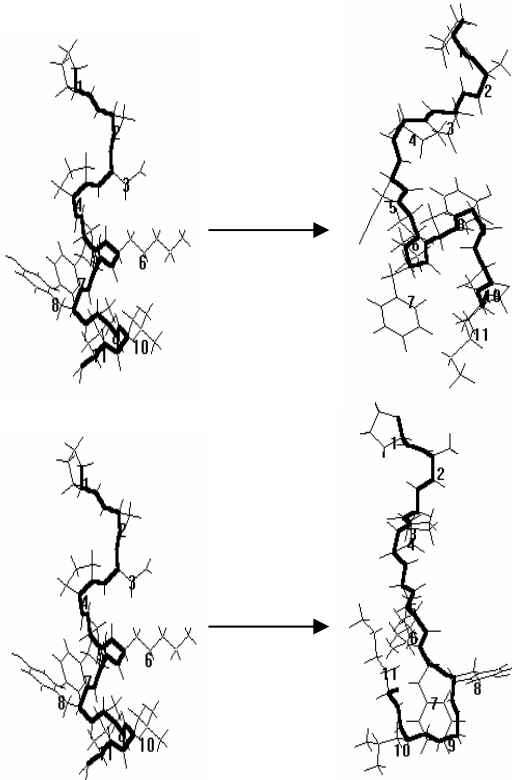


Fig.3. Conformational reorganization of the Physalaemin molecule at the molecular dynamic simulation in vacuum (at the top) and in water (below). The initial conformation take from [12].

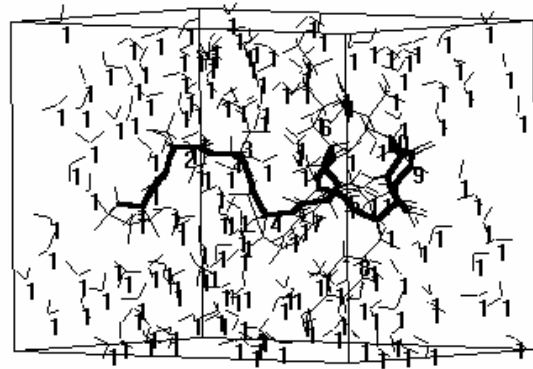


Fig.4. The final structure, received by means of MD simulations, after computer-optimization of the lowest-energy conformation of Physalaemin molecule in box with water molecules.

MD simulation for Physalaemin.

MD simulations for physalaemin during 300ps indicate that ψ angle for pGlu1 have a noticeable conformational flexibility. All side chains angles of Lys 6 were seen to be well-defined around 180° throughout the runs. Corresponding changes of dihedral angle values are presented in Table 2. The run with the all low energy starting struc-

Table 2. The permissible changes of values (in degrees) of ϕ, ψ, ω, χ dihedral angles of physalaemin lowest energy conformation under MD simulations in vacuum (upperline) and water (under line)

Residue	Backbone angles			Side chain angles				
	ϕ	ψ	ω	χ^1	χ^2	χ^3	χ^4	χ^5
pGlu ¹		111 to 117 115 to 120	190 to 188 185 to 189					
Ala ²	-100 to -110 -100 to -112	-69 to -52 -51 to -55	181 to 182 182 to 183	180 180				
Asp ³	-134 to -121 -120 to -125	93 to 89 97 to 100	172 to 180 180 to 175	-61(+2) -60(+2)	90 90			
Pro ⁴		-51 to -53 -40 to -42	185 to 180 175 to 182					
Asn ⁵	-58 to -68 -122 to -140	-34 to -45 157 to 165	187 to 189 176 to 180	181 to 188 -59 to -71	90 90			
Lys ⁶	-71 to -74 -95 to -100	-31 to -35 -96 to -99	180 to 178 182 to 185	182 (-2) 182	182 181	180 180	180 180	180 180
Phe ⁷	-60 to -65 -107 to -110	-43 to -44 154 to 160	179 to 180 182 to 183	179 179	90 90			
Tyr ⁸	-78 to -76 -112 to -114	-33 to -32 -46 to -47	-186 to -184 -186 to -182	-61 to -67 175 to 178				
Gly ⁹	-60 to -62 -62 to -63	-38 to -39 -36 to -39	-180 to -179 -180 to -178					
Leu ¹⁰	-82 to -83 -87 to -89	-63 to -62 -65 to -66	-174 to -175 -172 to -178	175 175	64 64	59 60	57 58	
Met ¹¹	-93 to -92 -65 to -68	-53 to -52 -54 to -52	-181 to -182 -181 to -184	-58 -59	180 180	181 180	180 180	

tures had an initial angles for Asn5 – Phe7 change its only around -10° and 10° . The conformational changes during the MD simulations in vacuum and in water are shown on Fig.3. As a result sinking the global structure of physalaemin in the box with water molecules was received the final optimized structure of peptide at the during the MD simulation (Fig.4). As can be seen from Table 2 the mobility of the Asp3-Lys6 amino acids stretch is considerably limited. So, the flexibility of residues in the 5th and 7th positions is limited by 10° as compared to the preceding part of molecule. This fact can be explained due to the important role of these residues in the formation of β -turn. Each angle varied about a single value, close to one of the set of possible angles calculated from molecular mechanics energy minimization [12].

Conclusion

We have carried out detailed analysis of the flexibility of the tachykinin molecules 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 Arg1-Pro4 sequence of substance P; (II). the α -helical conformation on Gln5- Met11 segment of peptide was more stabilized in vacuum, with the predominant hydrogen bonds NH(Phe⁸)-CO(Gln⁵), NH(Gly⁹)-CO(Gln⁶) and NH(Met¹¹)-CO(Phe⁷). than the extended conformations; (III) the similar molecular dynamics simulations for physalaemin indicated that relatively high stability of the low-energy conformations resulted not only from nonvalent interactions between residues but also from hydrogen bonds networks

NH(Lys⁶)...CO(Asp³), NH(Phe⁷)...CO(Pro⁴), NH(Gly⁹)...CO(Asn⁵), NH(Leu¹⁰)...CO(Lys⁶) and NH(Met¹¹)...CO(Phe⁷);

(IV) the β - turn conformation at the Asp3-Pro4-Asn5-Lys6 were more stabilized in vacuum and provide optimal nonvalent interactions between residues. The determined structures of these tachykinins may be used as the basis for the design of further peptidic selective antagonists.

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