Topographical Conformations of the Deltorphins Predicate δ Opioid Receptor Affinity

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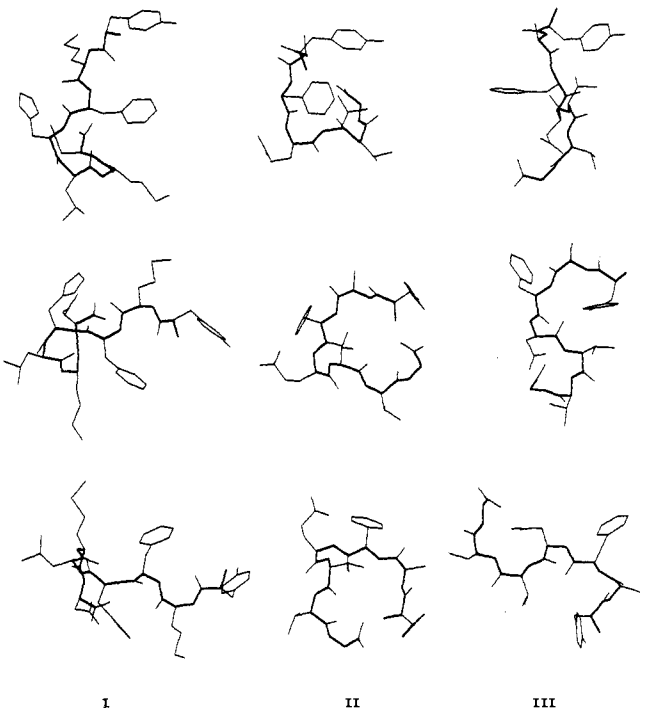
The use of molecular dynamics simulations to study biological and chemical processes in conjunction with bioactivity and receptor binding data provides meaningful information regarding structure–activity relationships. Garrett *et al.*[1] disclosed that a large quantity of relevant information is obtainable with PC programs that utilize simulated annealing optimizations and energy minimizations. If computer artifacts are minimized, these studies offer visual descriptions of molecules, facilitate comparative studies, and refine understanding of structural requirements in receptor interactions.

Emphasis on the molecular dynamics of opioid peptides was placed primarily on studies of enkephalins and enkephalin analogues,[2,10,11,17,18] and only preliminary computer modeling analyses of deltorphins were completed.[3,6,12,15,16] Deltorphins, amphibian skin peptides containing a D-amino acid at the second position,[4] are of interest because they exhibit higher affinity and selectivity for δ opioid receptors[4] than enkephalins or their derivatives.[4,5] Therefore, our study, based on ^{1}H-NMR[12] starting structures adapted molecular dynamics simulations[6] to generate several low-energy conformers and to compare the topographical features of solvated minimal-energy structures of the naturally occurring deltorphins: deltorphin A (H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2), deltorphin B (H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2), and deltorphin C (H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2).[7,13]



I II III

Figure 1. Three-dimensional representations of deltorphins A (I), B (II), and C (III), with *z*-axis perpendicular to the plane of the paper and alignment of the molecules with respect to Tyr[1] in the first row, D-Ala[2] in the second row, and Phe[3] in the third row. Molecular models were based on ^{1}H-NMR analyses by Amodeo *et al.*[12] and the simulated annealing schema of Tancredi *et al.*,[6] using HyperChem (v. 2.0, AutoDesk).

Our results revealed correlations between conformational differences of the three peptides and their receptor binding studies.[8,9,13] Low-energy models (Figure 1) illustrated that deltorphin B, which exhibits highest δ selectivity,[4,7,9] displayed a more compact structure than either deltorphin A or C; the compressed structures of deltorphin B exhibited higher energies than the other two peptides (Table I), suggesting that a compact structure, in contrast to an extended low-energy structure, may be preferred by the δ receptor. Low-energy dihedral angles (ϕ,ψ) and side-chain rotation angles (χ_1, χ_2) suggested that the position of the first three residues is integral to δ receptor association.[7,10]

Searching for δ receptor binding requirements, Keys *et al.*[11] proposed that analogues with high δ affinity bind to the receptor in a similar conformation and that low-energy conformers exist with optimal interaction at the receptor. While it is accepted that peptides adopt a similar conformation in binding to the δ receptor,[2,5,11] it is not clear if the δ receptor prefers low-energy conformers. Our data suggested that a higher energy conformer was favored by the δ receptor: several conformers of deltorphin B (−230.708 kcal/mol) and deltorphin C (−238.266 kcal/mol) exhibited higher energies and the highest δ selectivities ($K_i\mu/K_i\delta$ = 3122 and 1596, respectively),[9,13] while the conformers of deltorphin A (−289.002 kcal/mol) had the lowest energies and lower δ selectivity (839).[9,13]

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Table I. Molecular Dynamics Parameters for Low-Energy Conformers of Deltorphins A, B, and C

peptide low-energy conformers	residue	ϕ (deg)	ψ (deg)	χ_1 (deg)	χ_2 (deg)	energy (kcal/mol)	energy components (kcal/mol)					
							bond	angle	dihedral	van der Waals	H-bond	electrostatic
deltorphin A	Tyr		169.072	−69.412	−65.339	−289.002	1.625	30.17	16.894	−0.315	−3.415	−333.968
	D-Met	76.685	−84.239	153.053	−162.549							
	Phe	−35.122	135.061	−99.326	168.812							
	His	−131.42	−56.233	65.121	−150.672							
	Leu	−112.041	−163.977	−46.613	72.28							
	Met	−77.383	54.525	174.529	167.831							
	Asp	−99.343										
deltorphin B	Tyr		−51.716	74.052	−73.058	−230.708	1.629	7.622	7.297	−1.789	−1.538	−243.929
	D-Ala	−58.966	−61.978	172.605								
	Phe	−178.013	−160.412	−39.676	−61.563							
	Glu	−43.184	−31.465	39.486	174.582							
	Val	−40.162	124.733	−109.503								
	Val	−58.989	132.058	−104.583								
	Gly	93.374										
deltorphin C	Tyr		−70.851	53.965	−79.177	−238.266	1.767	8.039	7.356	8.829	−1.623	−262.634
	D-Ala	142.393	−72.759	166.034								
	Phe	−150.854	−105.503	19.813	−90.831							
	Asp	−51.927	147.897	−89.53	54.221							
	Val	−152.076	55.384	177.153								
	Val	−167.269	−54.019	71.071								
	Gly	−54.706										

Analysis of the energy components revealed variations in angle bending, dihedral, and van der Waals contributions (Table I). Deltorphin A exhibited a 4-fold greater angle bending and a 2-fold greater dihedral contribution than either deltorphin B or C. These variations resulted from flexibility of the residues in deltorphin A, facilitating deformations of bond angle and dihedral potentials, rendering accessibility to lower energy conformers than were attainable by either deltorphin B or C. Although differences in ϕ and ψ torsion angles were noted between all three peptides, deltorphin A exhibited distinct variations (Table I). Projection of these torsion angles revealed that deltorphin A was more extended in the C-terminal region than either deltorphins B or C. Deltorphin B displayed a reverse turn at the fifth residue with ϕ = −40.162° and ψ = 124.733° and could be exemplary of an optimal orientation for δ binding. This feature was absent in deltorphins A and C, in which backbone torsion angles were ϕ = =112.041° and −152.076° and ψ = −163.977° and 55.384°, respectively. Conformational studies of enkephalin analogues demonstrated that the δ receptor preferred folded over extended conformations.[10] This folded model, where the C-terminal region is oriented toward the N-terminus, suggests that the C-terminal region is critical for recognition by δ receptors.[6,8,13,14] Moreover, ^{1}H-NMR data revealed that in solution, the folded and extended conformers of both deltorphins A and C exist in equilibrium, providing evidence for the ability of these peptides to adopt folded conformations.[6,15] Thus, the δ receptor may selectively bind folded conformers.

The variation of van der Waals potential energies between deltorphin A (−0.345 kcal/mol), deltorphin B (−1.789 kcal/mol), and deltorphin C (8.829 kcal/mol) indicated the relevance of spatial orientation of the side chains and proximity of nonbonded atoms to low-energy conformations of the three deltorphins (Table I). Deltorphin C exhibited the largest van der Waals potential energy, indicating smaller interatomic distances between nonbonded atoms; the low-energy representation of deltorphin C revealed proximity between Asp^4 and the terminal amide of Gly^7 and between Asp^4 and the backbone carbonyl at Val^4 (Figure 1). The proximity of the atoms in these residues resulted in repulsive interactions that rendered higher van der Waals energy in deltorphin C than was observed in deltorphins A or B, where the side chains were positioned opposite each other in an alternating pattern, increasing the interatomic distances between the nonbonded atoms, thus decreasing van der Waals repulsions.

Deltorphin B formed an internal pocket with side chains oriented along the periphery of that pocket, and residues Tyr^1 and Phe^3 adopted alignments similar to deltorphin A (Figure 1). Spatial orientation of aromatic residues Tyr^1 and Phe^3 is critical for binding;[7,16,19] in particular, parallel orientation of these side chains in enkephalin analogues was required for δ receptor binding.[10] This orientation was expected for the low-energy deltorphin models; however, the low-energy representations revealed a parallel orientation between Tyr^1 and Phe^3 in deltorphins A and B but not in deltorphin C (Figure 1). Analysis of torsional parameters (χ_1, χ_2) suggested side chain rotational flexibility due to the variety of torsional parameters adopted by the low-energy conformers (Table I). Rotational flexibility of Tyr^1 in deltorphins A, B, and C was noted and similarly was recognized in studies with synthetic enkephalin analogues.[16,17] Phe^3 exhibited considerable diversity in side-chain orientations with χ_1 = −99.326°, −39.676°, and 19.813° for deltorphins A, B, and C, respectively (Table I). Since molecules are constantly undergoing translational, vibrational, and rotational movements, side-chain flexibility allows more conformations; thus it is feasible for the δ receptor to select conformers with parallel orientation of the aromatic residues, as displayed by the low-energy structures of deltorphins A and B (Figure 1).

Less rotational flexibility was observed at the crucial D-amino acid at position two: deltorphin A, χ_1 = 153.053°; deltorphin B, χ_1 = 172.605°; and deltorphin C, χ_1 = 166.034° (Table I). Spatial orientation of D-Ala^2 in deltorphins B and C revealed that the side chain appears on the same side as those of Tyr^1 and Phe^3 (Figure 1); D-Met^2 in deltorphin A is oriented on the opposite side. Our results with deltorphins supported the molecular dynamics simulation studies with enkephalin-derived peptides, which indicated that δ binding affinity was enhanced when the second residue was located on the same side as the first and third residues.[18]

In summary, molecular dynamics simulations are effective in determining topography of deltorphins at the δ receptor binding site and demonstrating that information from computer modeling simulations correlates with receptor binding data[4,7,13,19] and bioactivity studies.[4,14]

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Supplementary Material Available: Methods of data collection (1 page). Ordering information is given on any current masthead page.