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Communication

Molecular docking-based test for affinities of two ligands toward vasopressin and oxytocin receptors★✪☆

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Molecular docking simulations are now fast developing area of research. In this work we describe an effective procedure of preparation of the receptor–ligand complexes. The amino-acid residues involved in ligand binding were identified and described.

In recent years, conformationally constrained analogs of well-known bioactive peptides have acquired a growing importance in the effort to establish the relationships between the three dimensional structure and biological activity. Since the existing agonists and antagonists are still far from perfect, such knowledge could be of great help in the design of new, highly active ligands with improved selectivity. In pharmacological tests regarding the relation between structure of a specific bioligand and its bioactivity an important part take agonists and antagonists both peptide and nonpeptide, of human hormones: vasopressin and oxytocin (OT) — the subjects of our interest. In this work we have considered two potent selective V_{1a} receptor ($V_{1a}R$) antagonists: [Mca^1, $Tyr(Me)^2$]AVP and $DesGly^9$-[Mca^1]AVP, where Mca stands for β,β-cyclopentamethyleno-β-mer-

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☆This work consists of a test aimed at the verification of the potential usefulness of a new GPCR model proposed by Mosberg *et al.* (Univ. of Michigan, Ann Arbor, U.S.A.) in connection with the AutoDock program (Olson *et al.*, Scripps Research Institute, La Jolla, U.S.A.) for predicting affinities of a specific bioligand toward specific receptor.

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Abbreviations: Mca, β,β-cyclopentamethyleno-β-mercaptopropionyl.

captopropionyl, AVP – for arginine vasopressin (CYFQNCPRG-amide). [Mca^1, Tyr(Me)^2]AVP is also a partial V_2 receptor (V_2R) agonist.

METHODS

Starting models of new ligands and receptors were constructed using homology modeling. This part of our work involved usage of the available amino-acid sequences [1] in conjunction with a search within crystallographic database CSDS [2]. Three-dimensional (3D) models of our receptors: V_{1a}R, V_2R and oxytocin receptor, were built using the human opiate-γ receptor model proposed by Pogozheva and coworkers [3]. The missing 2nd and 3rd cytosolic loops and the extracellular N- and C-termini were built using the SYBYL [4] suite of programs. For verification of our 3D theoretical model of V_{1a}, V_2 and OT receptors (OTR) we have calculated the root mean square (RMS) deviation between our theoretical 3D structures and the X-ray crystal structure of rhodopsin at 2.8 Å resolution, which was published recently [5]. The result: 2.66 Å positively verifies our computer-modeled 3D structures. The next steps were: 1. preparation of full-atom models with atomic charges, 2. their relaxation and minimization (stand-alone and in the complexes with V_{1a}R, V_2R and OTR). All non-standard amino-acid residues included in both docked ligands were parameterized in accordance with the recommendations in the AMBER 5.0 [6] manual. Atomic net charges were optimized by fitting to the *ab initio* molecular electrostatic potentials (using the 6-31G* basis set in the GAMESS [7] molecular program package). Minimization of these models was done in AMBER 5.0 force field using constraints for transmembrane domains in V_{1a}R, V_2R and OTR models based on the opiate receptor models proposed by Pogozheva *et al.* [3]. The starting models of ligand-receptor complexes were prepared using the AutoDock 3.0 [8] program. These computer-docked and minimized models were subsequently used as the starting point in the discussion of the properties of newly-designed ligands and for the characterization of the binding sites of our receptors. Final complexes were selected based on the criterion of the internal ligand energy of the minimized receptor-ligand complexes. The amino-acid residues involved in ligand binding were identified and described. The docking procedure involved the AutoDock 3.0 program and its new and promising hybrid search technique that implements an adaptive global optimizer with local search. The global search method is an implementation of a modified genetic algorithm, with 2-point crossover and random mutation. The local search method is based on the optimization algorithm of Solis & Wets [9], which has the advantage that it does not require gradient information in order to proceed – as was the case in previous versions of AutoDock. It also uses fixed variances for the determination of the probabilistic way of a change of a particular state variable, like the x-translation. These variances are either doubled or halved during the search, depending on the number of consecutive successful or failed moves. Success is a drop in energy. Receptor-ligand complexes were relaxed and minimized by the consecutive use of the minimization and constrained simulated annealing (CSA) protocols *in vacuo* (in accordance to the AMBER 5.0 manual [6]) with all but the transmembrane-domain C^{α} atoms free to move. This was done in the AMBER 5.0 force field. Sample complexes of docked ligands are presented in Fig. 1.

RESULTS AND DISCUSSION

For CSA refinement only those complexes were retained whose receptor-ligand interaction energy was about 1000 kcal/mole or less above the absolute minimum. The AutoDock force field used in the docking procedure is very limited in its functionality – it uses electrostatic interactions and van der Waals potential. This 1000 kcal/mole-criterion eliminated all complexes which were not properly minimized by AMBER. For efficient relaxation of the remaining part of complexes we used CSA with heating the environment up to 1200 K (1 ps), keeping this temperature constant (2 ps) and re-cooling to low temperatures (12 ps of CSA) – as shown in Fig. 2. Having this

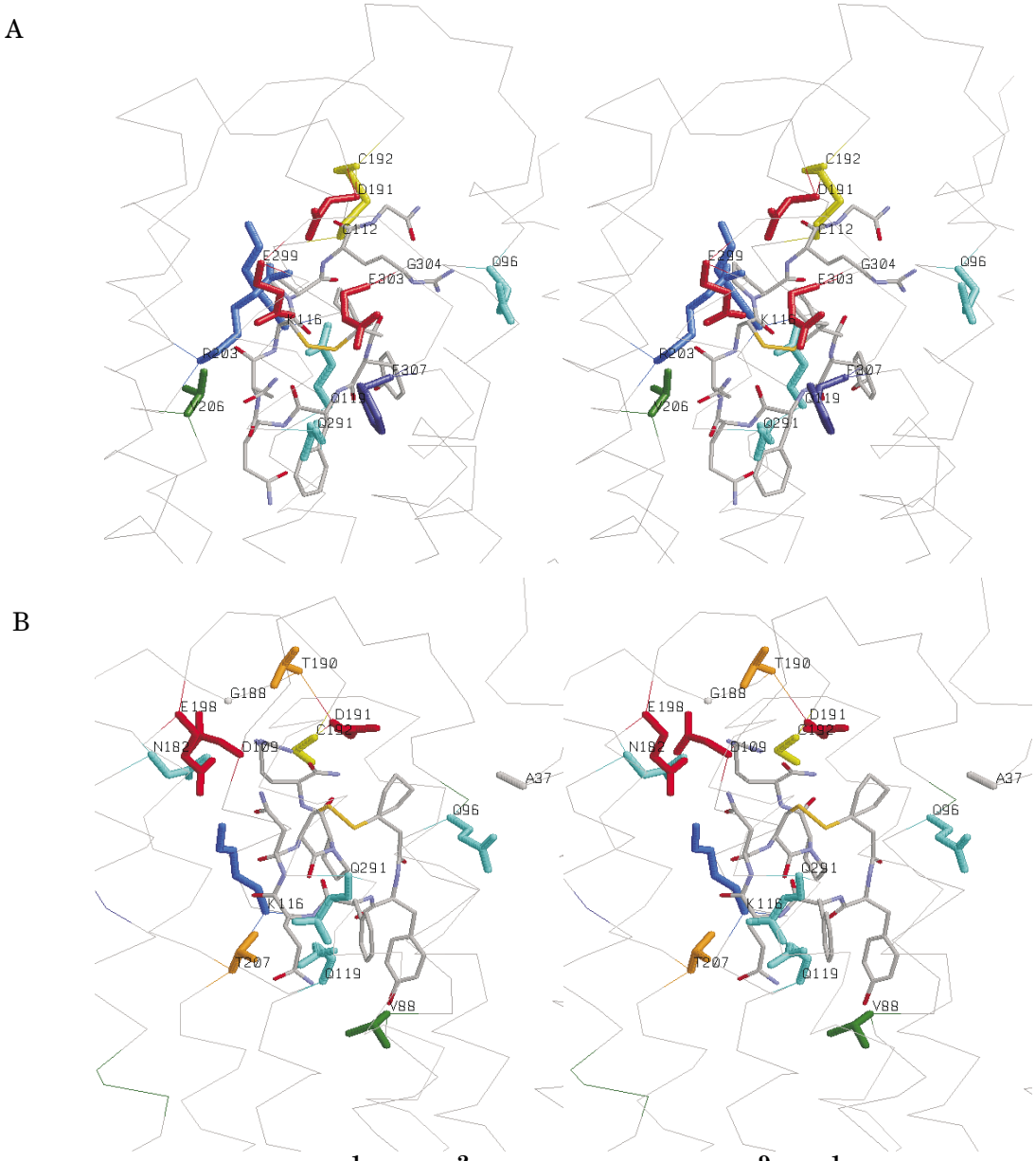

Figure 1. Stereoview of ligand Mca^1,Tyr(Me)^2]AVP (panel A) and DesGly^9-[Mca^1]AVP (panel B) docked in human V_2 receptor.

The amino-acid residues responsible for ligand binding are colored according to their chemical properties. Colors of polar amino-acid residues are brighter than non-polar. We used standard RasMol (Sayle, R., *RasMol V2.6, Molecular Visualisation Program,* Glaxo Wellcome Research and Development, Stevenage, Hertfordshire, U.K.) color coding of all receptor amino-acid residues.

done we have selected, based on the energetic criterion, 5–7 complexes of [Mca^1, Tyr(Me)^2]- AVP and DesGly^9-[Mca^1]AVP in each of the $V_{1a}R$, V_2R and OTR receptors. Their final energies are presented in Table 1. Receptor amino-acid residues responsible for binding new ligands were identified on the basis of the distance criterion. The residues whose any atom is not further than 4.5 Å away from all accepted conformations of our ligands are shown in Table 2 and arranged so that the more often a particular residue is involved in binding the ligand the higher is the position it occupies in the table.

Not in line with our expectations both ligands were docked relatively shallow in the binding site of the V_{1a} receptor – close to the extracellular loops. Their minimization and relaxation did not radically change their position, which suggests

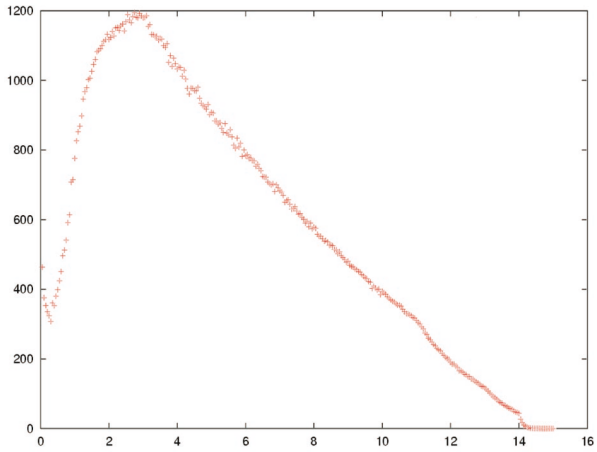

Figure 2. Changes in temperature during the constrained simulated annealing (CSA).

This sample drawing presents changes in temperature during CSA of DesGly^{9}-[Mca^{1}]AVP ligand in OT receptor.

the optimal docking. Ligands in complexes with the other two receptors (V_2R and OTR) were docked in the midst of the docking nest on the extracellular sides of the receptors. This nest, surrounded by the helices TM3-TM7 is bordered with the extracellular loops on its top and (in $V_{1a}R$, V_2 and OTR, respectively) with: TM2: P(107,95,95); TM3: M(135,123,123); TM5: V(217,206,208), T(218,207),Y(209); TM6: W(304,285,288), F(308,289,291), F(309,290,292); and TM7: A(334),M(311,315) on their bottom. Both our ligands being rather small molecules (93 and 97 atoms) had the advantage of free movements within the receptors' pockets but their positions did not change much even after a relatively hot (up to 1200 K) CSA, despite allowing unconstrained flexibility to all TM side chains, both ligands and the receptors' loops. This suggests a very good implementation of the genetic algorithm in the docking procedure even though the ligand–receptor interaction energies were extremely high. Slight differences between the conformations of the two ligands complexed to the three receptors studied (especially between V_{1a} and V_2 receptors) show that the CSA protocol caused good relaxation of ligands and found very good minima.

Table 1. Energies of ligand–receptor complexes [kcal/mole]

[Mca^{1}, Tyr(Me)^{2}]AVP			DesGly^{9}-[Mca^{1}]AVP		
$V_{1a}R$					
Ligand code	Energies after docking	Energies after CSA	Ligand code	Energies after docking	Energies after CSA
t1	-35.46	-6557.85	q2	-1327.53	-6411.98
t9	-2592.98	-6818.73	q8	-3274.14	-6837.58
t10	-2976.87	-6688.76	q14	-1672.87	-6838.42
t15	-4167.18	-7000.76	q17	-3184.72	-6723.41
t19	-1154.91	-6486.88	q18	-2768.16	-6819.06
t26	573.54	-6387.14	q19	-1748.36	-6822.32
t29	-2093.19	-6743.20	q25	-484.80	-6769.22
			q28	-1879.31	-6820.07
V_2R					
t1	14830.40	-4241.39	q1	10244.70	-4296.14
t4	23073.61	-3746.76	q2	8961.29	-4505.76
t6	12783.58	-4080.66	q20	11157.45	-4071.46
t10	16789.64	-4094.84	q25	10184.93	-4343.22
t12	12645.02	-4175.17	q30	7397.31	-4282.68
t13	15055.40	-4204.38			
t25	16749.78	-4062.96			
OTR					
t2	616.40	-3759.38	q1	-679.90	-5384.34
t5	65.08	-5149.09	q2	-1625.11	-5690.12
t7	3267.49	-4786.22	q4	-1682.78	-5193.44
t17	-1149.03	-5514.38	q14	-1599.62	-5534.25
t22	-1008.45	-5268.44	q15	723.59	-5045.37
t27	2821.54	-4402.22	q20	-1297.94	-5612.01
t28	1467.92	-3786.84			

Table 2. Amino-acid residues forming binding pockets of the receptors

[Mca^1, Tyr(Me)^2]AVP			DesGly^9-[Mca^1]AVP		
$V_{1a}R$	V_2R	OTR	$V_{1a}R$	V_2R	OTR
Thr333	Glu303	Gln119	Gln311	Gln119	Asp186
Thr218	Gln119	Lys116	Thr218	Gln96	Gly183
Ile330	Gln291	Cys112	Ala334	Gln291	Gln119
Phe307	Asp191	Ala308	Ile330	Glu198	Ala308
Asn327	Lys116	Asp186	Asn327	Cys192	Thr202
Gln311	Cys112	Gln96	Thr333	Asp191	Lys198
Thr221	Phe307	Ile312	Phe307	Thr190	Lys116
Arg214	Gly304	Ile201	Thr221	Gly188	Glu307
Val217	Glu299	Lys198	Trp304	Asn182	Gln295
Glu326	Val206	Gly196	Arg214	Ala37	Gly196
Met312	Arg203	Asp182	Thr331	Lys116	Trp195
Phe308	Cys192	Val120	Phe308	Val88	Cys187
Cys203	Gln96	Gln92	Val217	Asp109	Cys112
		Phe91	Cyx203	Thr207	

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