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QUARTERLY

Molecular modeling of the oxytocin receptor/bioligand interactions★°

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Oxytocin is a nonapeptide hormone (CYIQNCPLG-NH_2, OT), controlling labor and lactation in mammalian females, *via* interactions with specific cellular membrane receptors (OTRs). The native hormone is cyclized *via* a 1–6 disulfide and its receptor belongs to the GTP-binding (G) protein-coupled receptor (GPCR) family, also known as heptahelical transmembrane (7TM) or serpentine receptors. Using a technique combining multiple sequence alignments with available experimental constraints, a reliable OTR model was built. Subsequently, the OTR complexes with a selective agonist [Thr^4,Gly^7]OT, a selective cyclohexapeptide antagonist L-366,948 and oxytocin itself were modeled and relaxed using a constrained simulated annealing (CSA) protocol. All three ligands seem to prefer similar modes of binding to the receptor, manifested by repeating receptor residues which directly interact with the ligands. Those involved in the three complexes are putative helices: TM3: R113, K116, Q119, M123; TM4: Q171, and TM5: I201 and T205. Most of them are the equivalent residues/positions to those found in our earlier studies, regarding related vasopressin V2 receptor/bioligand interactions.

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Abbreviations: AVP, [Arg^8]vasopressin; CSA, constrained simulated annealing; GPCR, G protein-coupled receptor; OT, oxytocin; OTR, oxytocin receptor; V2R, vasopressin V2 receptor; V1a(b)R, vasopressin V1a(b) receptor; 7TM, heptahelical transmembrane receptor or domain; VP, vasopressin; TM3-TM7, putative helices.

The nonapeptide hormone oxytocin (CYIQNCPLG-NH_2, OT) consists of a 20-membered cyclic hexapeptide, the tocin ring ensuing from a disulfide bond between Cys^1 and Cys^6, and of a C-terminal tail, comprising the Pro^7-Leu-Gly^9 amide. Oxytocin regulates labor and lactation in mammalian females, *via* interactions with specific cellular membrane receptors (OTRs). OTR belong to the superfamily of over 1000 of, both functionally and structurally homologous, GTP-binding (G) protein-coupled receptors (GPCRs). GPCRs, also known as heptahelical transmembrane (7TM) or serpentine receptors, are the integral proteins of the cell membrane and form the largest known family of homologous proteins. Despite an exceptional diversity in primary signals for various GPCRs (ranging from photons, neurotransmitters, peptide hormones, nucleosides and nucleotides, eicosanoids, metal ions, to glycoprotein hormones [1]), all receptors utilize the same transduction mechanism, implicating a cytosolic heterotrimeric GTP-binding (G) protein in mediation between a GPCR and an intracellular target effector. The latter are various enzymes or ion channels, generating secondary intracellular signals, virtually controlling all cell activities [2]. The structural homology among GPCRs consists of a common heptahelical transmembrane domain (7TM), exhibiting also a considerable sequence homology [3, 4]. Although no details on the GPCR architecture are known at the atomic resolution level, a recent significant progress, including both the low (6 Å) resolution structure of rhodopsin [5, 6] and the multi-sequence analysis [3, 4], prompted the development of perhaps the most rational strategy for the GPCR modeling [4, 7, 8]. It consists of a multi-sequence alignment of some 200–500 representative GPCRs resulting in a statistically balanced probe for averaged distributions of polar/non-polar and conservative/non-conservative residues, thus allowing a choice of the putative helices TM1-TM7 along the sequence. Subsequently, TM1-TM7 are threaded onto the low-resolution structure of rhodopsin [6], with simultaneous meeting of most of the experimental constraints [4, 7], and then refined to the self-consistency of 1.67 Å. The automated GPCR-modeling server, based on this scheme, is available *via* Internet [8]. The resulting GPCR template, even if chiefly applying to rhodopsin, provides a good start for 7TM models of other receptors, given the sequence homologies among various GPCR subfamilies [9].

In this work we intend, using molecular modeling, to analyze possible docking modes in OTR of oxytocin, its selective agonist [Thr^4, Gly^7]-oxytocin and its selective cyclohexapeptide antagonist L-366,948. The latter is a productive chemical modification of an L-365,209 isolated from the bacteria *Streptomyces silvensis* [10]. The affinity data [10, 11] and the structures of the ligands are given in Table 1.

METHODS

The 7TM domain of the human OTR was obtained from the Swiss-Model protein modeling server [8], given the helix alignments in agreement with those proposed by Baldwin [3, 12]. The loops and the amino domain were built using the SYBYL suite of programs [13]. Initial ligand docking was attained in several ways, taking into account the complementarity of the electrostatic potentials in the OTR extracellular pocket (vide infra) and around the ligand. The systems were relaxed by the consecutive use of minimisation and constrained simulated annealing (CSA) protocols *in vacuo*, with all but the 7TM C^α atoms free to move. Optimal ligand docking modes were selected from a critical comparison of the ligand/receptor interaction energy terms (as those given in Fig. 1) against conclusions on biologically relevant OTR residues arising from structure-activity data. In particular, care was taken for the hydrophobic N-terminus of oxytocin to sit relaxed on the hydropho-

bic floor in the receptor's extracellular pocket [14] and simultaneously to maintain a hydrophobic OT Leu^8/OTR Phe^{108} contact, believed to be the driving force for the receptor/agonist selectivity [15, 16]. All non-standard amino-acid residues were parameterized as recommended in the AMBER 4.1 manual [17]. In particular, the charges were optimized by fitting them to the *ab initio* molecular electrostatic potentials (6-31G* basis set, GAMESS molecular orbital program package [18]) for several conformations of each new residue, followed by consecutive averaging the charges over all conformations, as recommended in the new RESP protocol [19]. Initial structures were relaxed using a constrained simulated annealing (CSA) protocol as described in Table 2 in Ref. [14]. Details of the modeling and computations are also described in Ref. [14] for homologous vasopressin (VR) antidiuretic receptor (V2R)/agonist systems. Computations were done using AMBER 4.1 suite of programs [17] on SGI Power Challenge 8xR10000 and/or SGI Origin 2000 4xR10000 computers. The images for presentation were prepared using the MolMol program [20].

RESULTS AND DISCUSSION

Any GPCR modeled to the rhodopsin template [7, 8] has about 20 Å deep and about 14 Å wide hole on the extracellular side, nested between the transmembrane helices TM3- TM7, with a narrower extension towards TM2. In OTR this cavity ends with a floor made of mainly hydrophobic largely conservative residues TM3: M123, TM5: V208, Y209 and TM6: W288, F291 and F292, having their counterparts in other GPCRs from the common rhodopsin family, in particular those of OT/VP subfamily [14]. The hole is big enough to accommodate the tocin ring of oxytocin. In view of extensive sequence homologies between oxytocin and vasopressin on the one hand, and among their receptors on the other, it is reasonable to assume common or largely similar docking modes [14, 21, 22] for the hormones and analogs in their respective receptors.

These were the starting assumptions, for the structures of the relaxed complexes given in Fig. 1. Clearly, both the native hormone (Fig. 1A) and the agonist [Thr^4,Gly^7]OT (Fig. 1B), and the cyclohexapeptide antagonist (Fig. 1C) remain in the OTR interior despite relatively harsh treatment [14] during the CSA relaxation.

The analysis of the interaction maps (Fig. 2 A, B, C) enabled us to single out the receptor residues potentially important in ligand binding. Regarding the OTR-OT complex, the following OTR residues are good candidates for interactions with OT: TM3: R113 interacting with N5, C6 and P7 of OT, K116 with C6, Q119 with C1 and M123 with C1 and Y2. In TM4: Q171 with Y2,Q4; in TM5: I201 with I3,Q4, T205 with Y2,I3 and V208 with Y2; in TM6: W288 with C1,Y2 and Q295 with I3,N5,C6 (see Fig. 2A).

Similarly, the following OTR residues have been singled out as potentially interacting with [Thr^4,Gly^7]OT: for TM3: R113 with T4,C6,G7,L8, V115 with L8, K116 with T4,L8, Q119 with C1, M123 with C1,Y2 and S126 with C1; TM4: Q171 with T4; TM5: T200 with T4, I201 with T4, T205 with Y2,I3, V208 with Y2 and Y209 with Y2; TM6: W288 with C1,Y2 and Q295 with I3,N5,C6 (see Fig. 2B).

Finally, the following OTR residues have been singled out as potentially interacting with L-366,948: for TM3: R113 with H6, V115 with NA2, K116 with I3,H6, Q119 with NA2,DP4 and M123 with DP4; TM4: Q171 with LP5,H6; TM5: I201 with LP5,H6, I204 with LP5 and T205-LP5; TM6: W288 with I3 and Q295 with P1 (see Fig. 2C).

In addition, all three bioligands interact with residues from the second extracellular loop. Specific contacts involve F191-P7,L8, Q193-Q4 in the OTR-OT complex (Fig. 2A); F191-G7, Q193-G7 in OTR-[Thr^4,Gly^7]OT (Fig. 2B); and F191-NA2 and I192-NA2,H6 in OTRL-366,948 (Fig. 2C).

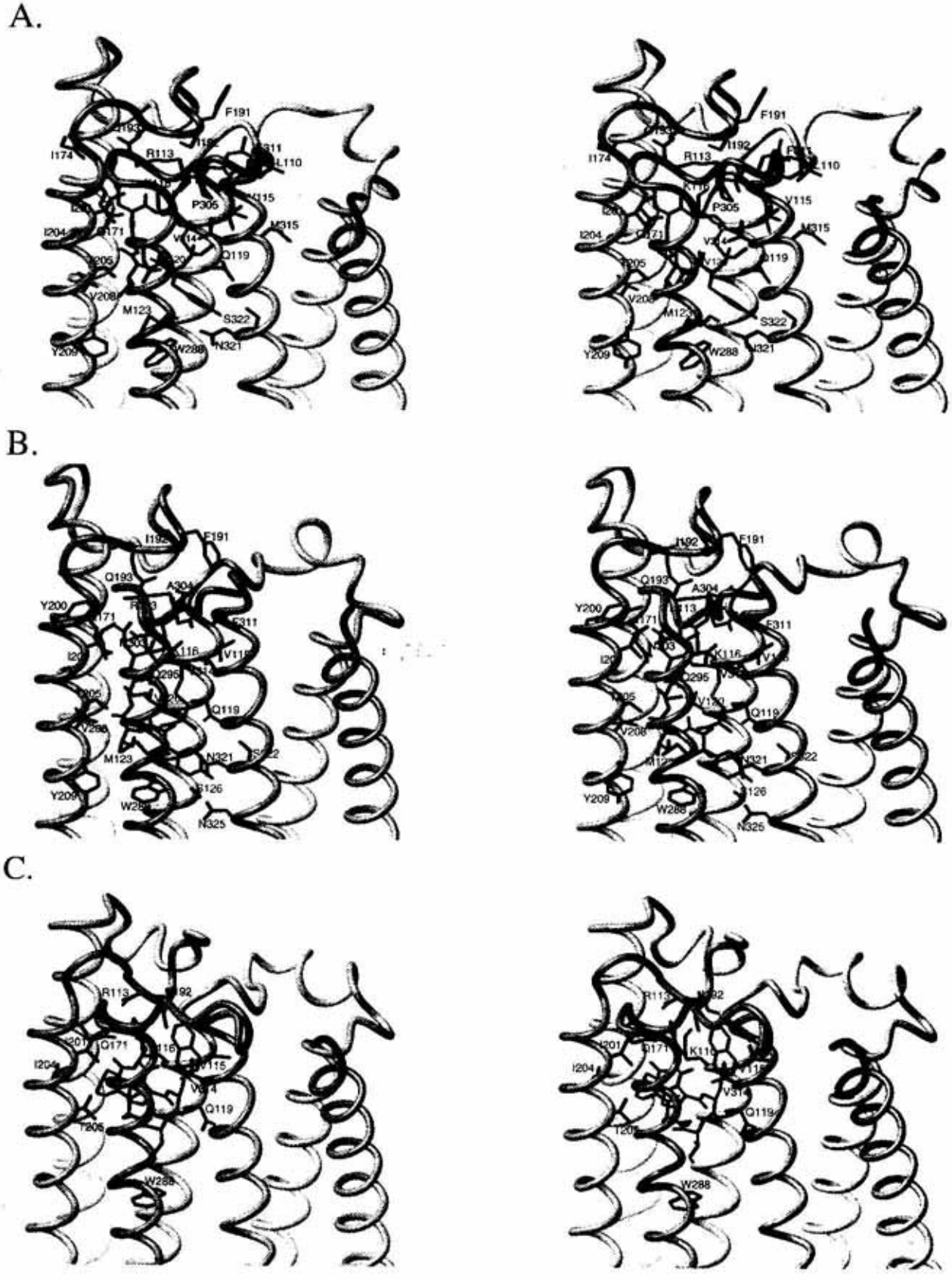

Figure 1. Stereodiagrams of the relaxed docking modes. Only the receptor extracellular halves are exposed.

Table 1. Affinity of oxytocin, arginine-vasopressin and two OTR to cognate receptors.

The affinity constants K_d are bold-faced for **agonists,** italicized for *antagonists* and unaltered if biological activity is unknown.

Ligand[a]	K_d [nM]			
	V1aR	V1bR	V2R	OTR
Vasopressin, AVP: CYFQNCPRG-NH_2, (1-6)-SS- link	**1.7**	**3.2**	**0.4**	**1.6**
Oxytocin, OT: CYIQNCPLG-NH_2, (1-6)-SS- link	**56**	**251**	**89**	**1.9**
[Thr^4,Gly^7]OT	3700		>10000	**1.0**
L-366,948: cyclo(Pro-D-NA-Ile-DP-LP-D-His)	*2600*[b]		*910*[b]	*2.2*[b]

[a]See Ref. [10]; [b]see Ref [11]. Abbreviations: NA, β-napthylalanine; DP, D-piperidinecarboxylic acid; LP, L-piperidinecarboxylic acid.

It can be seen that both the agonist and antagonist OTR/peptide complexes exploit nonpolar interactions between the ligands' N-terminal 1-3 amino acid triad and the hydrophobic floor at the bottom of the OTR extracellular cavity, and simultaneously develop numerous polar and nonpolar interactions with the walls of the cleft.

The obtained results are preliminary and the suggested interactions should be considered only good starting hypotheses for the mutagenesis-affinity studies, which would confirm or contradict them. This work is also preliminary in that that the simulations were carried out *in vacuo*, with the positional constraints imposed on all the 7TM C^{α} carbons, to prevent the receptors from melting due to harsh treatment, imposed by and typical of the simulated annealing. In this way, on the one hand, a resemblance of the resulting complexes to

Figure 1. Stereodiagrams of the relaxed docking modes. Only the receptor extracellular halves are exposed.

OTR (in light blue) is shown schematically as its backbone trace only, except for the residues interacting with the ligand, which are labeled and their side chains are shown in dark blue. The ligands are shown in green, with their atoms in standard colors. Panel A: OTR-OT. Panel B: OTR-[Thr^4,Gly^7]OT. Panel C: OTRL-366, 948. The human OTR sequence, with the putative TM helices underlined, supplements the Figure.

MEGALAANWS AEAANASAAP PGAEGNRTAG PPRRNEALAR VEVAVLCLIL LLALSGNACV 60

LLALRTTRQK HSRLFFFMKH LSIADLVVAV FQVLPQLLWD ITFRFYGPDL LCRLVKYLQV 120

VGMFASTYLL LLMSLDRCLA ICQPLRSLRR RTDRLAVLAT WLGCLVASAP QVHIFSLREV 180

ADGVFDCWAV FIQPWGPKAY ITWITLAVYI VPVIVLATCY GLISFKIWQN LRLKTAAAAA 240

AEAPEGAAAG DGGRVALARV SSVKLISKAK IRTVKMTFII VLAFIVCWTP FFFVQMWSVW 300

DANAPKEASA FIIVMLLASL NSCCNPWIYM LFTGHLFHEL VQRFLCCSAS Y

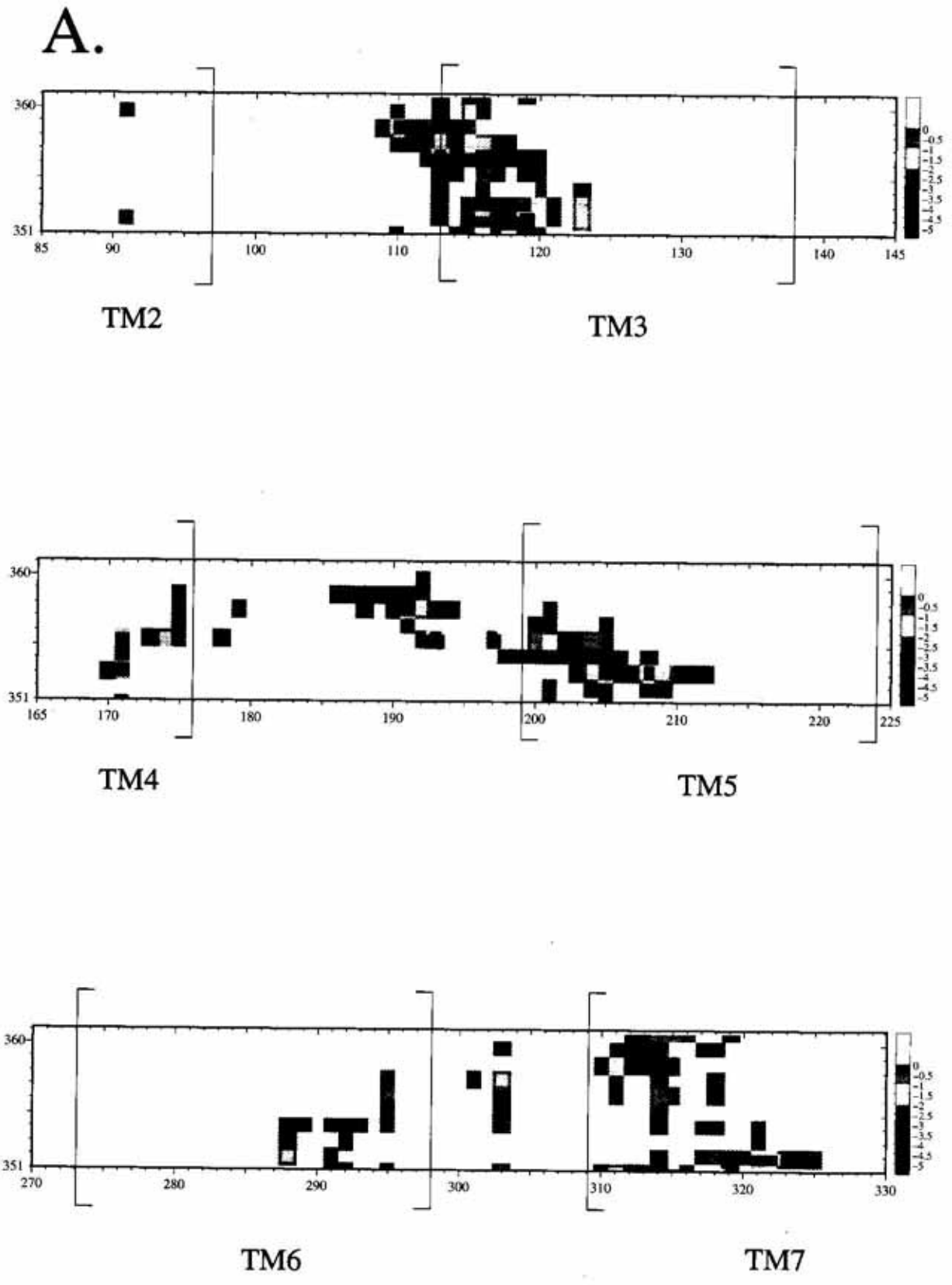

Figure 2A.The maps of the receptor-ligand interactions for the relaxed structures.

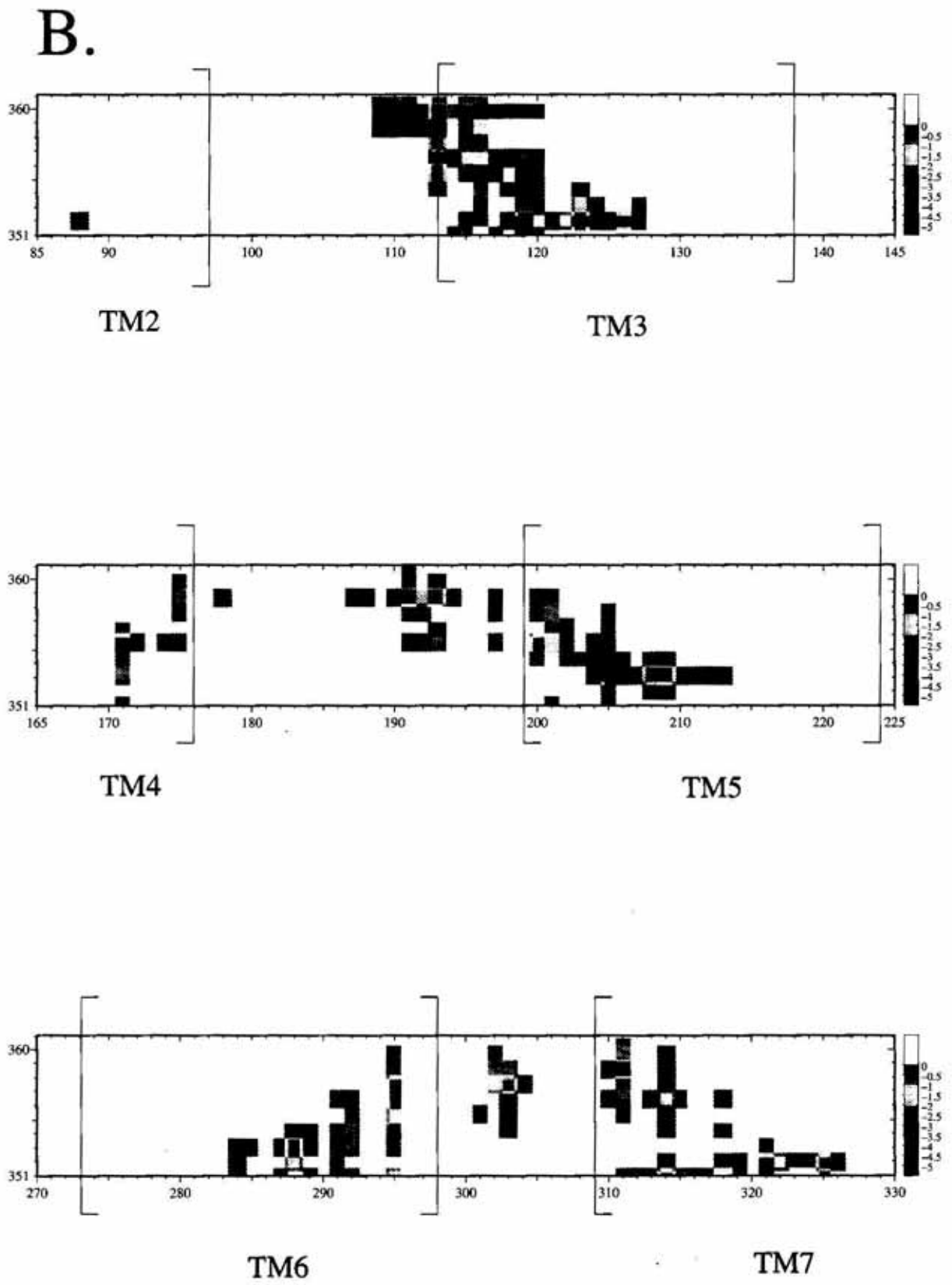

Figure 2B.The maps of the receptor-ligand interactions for the relaxed structures.

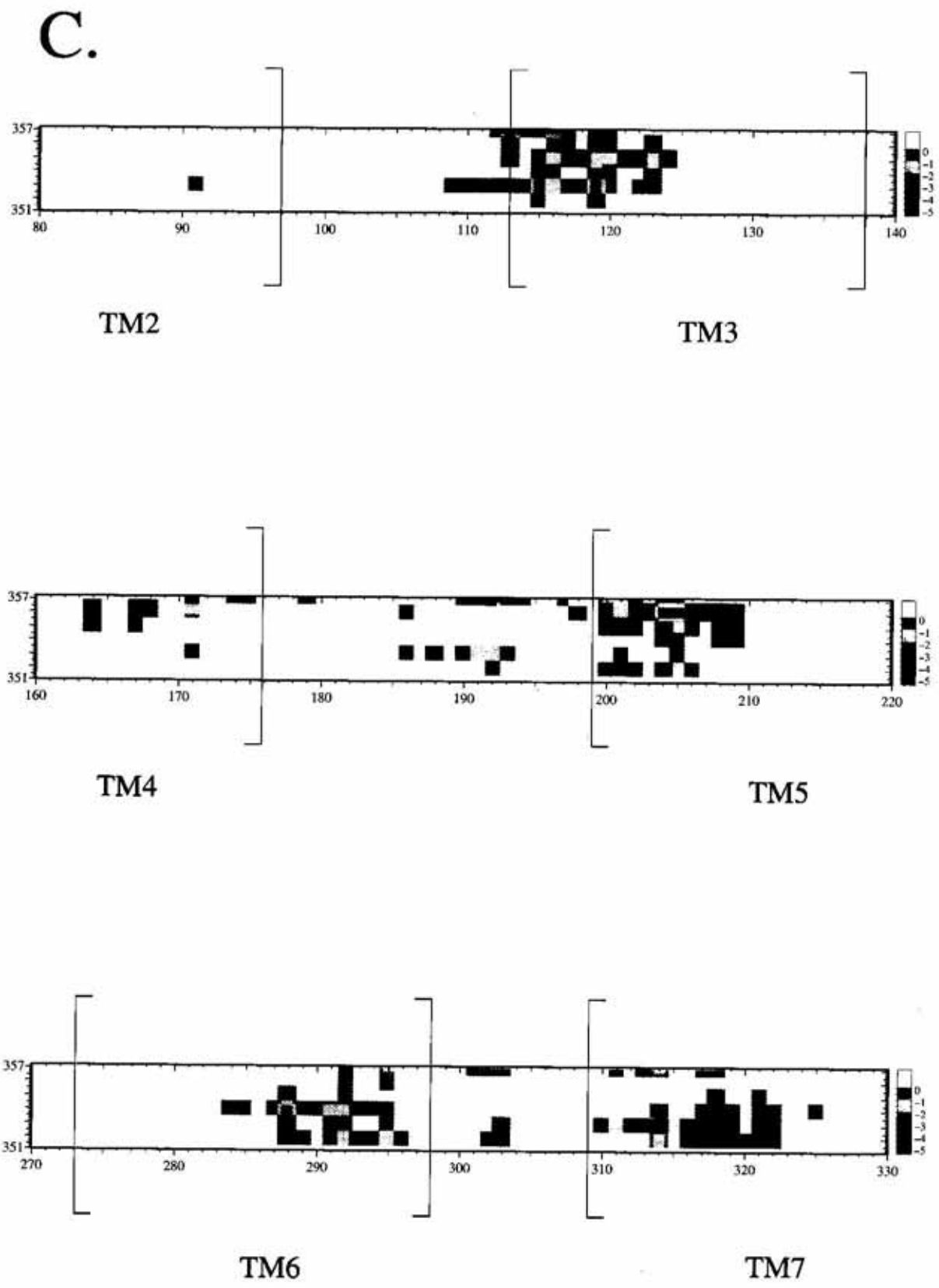

Figure 2C. The maps of the receptor-ligand interactions for the relaxed structures.

the low-resolution experimental images of rhodopsin [5, 6] was purposefully preserved, and, on the other hand, the complexes present excellent starting structures for unconstrained simulations in a model hydrated phospholipid bilayer, now being in progress.

It is worth mentioning that our results on agonist docking agree with those obtained by Mouillac *et al.* [23] for a related AVP/V1aR system. Furthermore, some of the equivalent V1aR residues have already been found critical for the ligand affinity.

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Figure 2. The maps of the receptor-ligand interactions for the relaxed structures.

The contours represent the interaction energies for specific pairs of amino-acid residues from the receptor and the ligand. The energy of the interactions (kcal/mol) is defined in the color scale on the right. Horizontal axis: successive residues of OTR, with the sequences interrupted in the regions with no interactions to the ligand. Vertical axis: ligand amino-acid sequence (numbered 351–360 for OT and [Thr^4,Gly^7]OT, and 351–356 for L-366,948). Panel A: OTR–OT. Panel B: OTR–[Thr^4,Gly^7]OT. Panel C: OTRL–366,948.

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