Acta Biochimica Polonica

Vol. 48 No. 4/2001

1203–1207

QUARTERLY

Communication

Signal transmission *via* G protein-coupled receptors in the light of rhodopsin structure determination★✪

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Received: 17 October, 2001; accepted: 12 November, 2001

Key words: GPCRs, neurohypophyseal receptors, molecular modeling, signal transduction

G protein-coupled receptors (GPCRs) transducing diverse external signals to cells *via* activation of heterotrimeric GTP-binding (G) proteins, estimated to mediate actions of 60% of drugs, had been resistant to structure determination until summer 2000. The first atomic-resolution experimental structure of a GPCR, that of dark (inactive) rhodopsin, thus provides a trustworthy 3D prototype for antagonist-bound forms of this huge family of proteins. In this work, our former theoretical GPCR models are evaluated against the new experimental template. Subsequently, a working hypothesis regarding the signal transduction mechanism by GPCRs is presented.

The newly published structure of dark rhodopsin (RD) at 2.8 Å resolution [1] provides for the first time ever a detailed view of intramolecular links and pivots typical of the inactive state of RD. These features, when critically limited to a selection of conservative residues, are likely to be valid for a general antagonist-bound receptor state and pertinent to a general signal transduction mechanism, typical of the whole RD-like G protein-coupled re-

★Presented at the XVI Polish Peptide Symposium, September 1–4, 2001, Jagiellonian University, Kraków, Poland.

✪This work was supported by the State Committee for Scientific Research (KBN, Poland) grants 6 P04A 024 17 (JC), 7 T09A 006 20 (PD), 7 T09A 138 21 (AG), and by the Academic Computer Center in Gdańsk TASK, regarding the use of the SGI Power Challenge 8xR10000 and/or Origin2000 4xR10000 supercomputers.

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Abbreviations: G, GTP-binding; GPCRs, G protein-coupled receptors; MD, molecular dynamics; RD, dark rhodopsin.

ceptor (GPCR) family. However, it is important to simultaneously realize that the experimental structure of dark RD includes an extracellular β-plug (combined from 2+2 β strands from the N-terminus and the 2nd extracellular loop, respectively), blocking deeply the exit from the RD ligand pocket. This feature seems unlikely in most other GPCRs, with ligands reversibly binding and leaving in milliseconds [2].

METHODS

Standard techniques for protein multiple sequence alignment [3] were employed, see e.g. Fig. 1 in [4] for sample usage. Protein homology modeling as implemented in Sybyl [5] and molecular dynamics (MD) as implemented in AMBER 5.0 [6] were used for the construction and relaxation, respectively, of the antagonist-bound forms of the neurohypophyseal vasopressin V1a, V2 and oxytocin receptors (V1aR, V2R and OTR, respectively), using the experimental RD structure (file 1f88 in the Brookhaven Protein Data Bank [7]) as a template. A home-made program for studying intra- and intermolecular interactions was used. Figures 1 and 3 were prepared using the Swiss PDB Viewer program [8] and Fig. 2 using the PlotMTV [9] program.

RESULTS AND DISCUSSION

Multiple sequence alignment applied simultaneously to human RD, V1aR, V2R, OTR and to four human opiate receptor sequences confirmed the occurrence of amino-acid residues that are conserved or similar over the whole RD-like GPCR family [10, 11]. They are collected in Table 1.

Prior to the V1aR, V2R and OTR model building, we compared our former favorite theoretical [11] with the experimental [1] RD model, Fig. 1. Both templates were relaxed by MD before overlapping. To our surprise, the transmembrane helices TM1-TM4 (rear) and TM7 (front-right) fit quite well in both models. TM5s fork away to the top, the experimental one more strongly sticking out of the bundle to reach 6–7 Å displacement at the N-termini. Transmembrane helices 6 are shifted relative to each other by a turn, the experimental being closer to the cytosol. Furthermore, a detailed inspection of the overlapping structures (not shown) indicates that over 50% of the intra-bundle side chains are also in similar conformations and fit well one another in both models. Thus, despite its obsoleteness, our to date favorite template, adopted from the theoretical model of Pogozheva *et al.* [11], has proven an excellent 3D prediction of a GPCR structure. Consequently, the conclu-

Table 1. Twenty-five conservative residues ensuing from multiple sequence alignment (see, e.g., [4]), in the order RD/OTR/V1aR/V2R.

These residues are potential candidates for a net of conserved allosteric interactions vital to signal transduction [10,11].

TM1	TM2	TM3	TM4	TM5	TM6	TM7
N55/57/69/55	N78/H80/92/80	A124/S126/138/126	W161/161/175/164	C222/219/C235/224	V254/T277/293/273	S298/N321/340/317
T58/V60/72/58	A82/84/96/84	L128/130/142/I130		Y223/220/236/Q225	F261/284/Y300/280	A299/S322/341/318
	D83/85/97/85	L131/M133/145/133			C264/287/303/283	N302/325/344/321
		E134/D136/148/136			W265/288/304/284	P303/326/345/322
		R135/137/149/137			P267/290/306/286	Y306/329/348/325
		Y136/C138/Y150/H138			Y268/F291/307/287	

sions of our former papers, where the old template was used as a starting point for any further considerations, should not be totally out of date, either [4, 12].

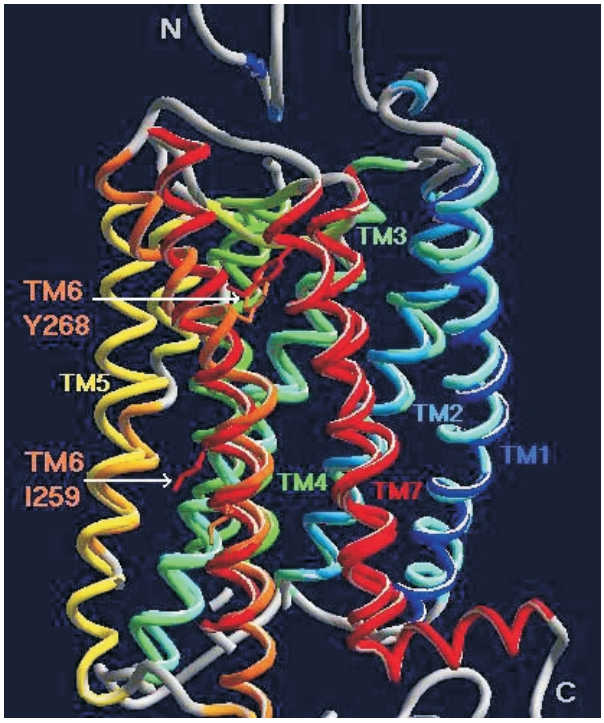

Figure 1. The old human RD theoretical template [11] (darker) optimally superposed onto the new experimental one [1] (ligter).

Prior to the superposition both models were relaxed using MD. They are colored according to sequence progression from intensive blue (the extracellular N-terminus) to intensive red (the C-terminus). The TM1–TM7 helices are color-labeled accordingly. The C^{α}-based RMS for 132 TM core atoms equals 1.53 Å. TM1–TM7 run counterclockwise when top-viewed. It is seen that TM1–TM3 and TM7 overlap excellently (the RMS values between 1–2 Å, not shown), TM5 diverge significantly to get about 6–7 Å apart of each other at the extracellular bilayer-water interface (the new TM5 leaning more outside of the bundle), TM4 (rear) and TM6 (front) fit moderately well (the RMS values between 2–3 Å, not shown), however, the new experimental template lies almost exactly a helical turn closer to the cytosol than the old theoretical one, e.g. compare the positions of I259 and Y268.

The V1aR, V2R and OTR inactive receptors were homology-modeled from the experimental dark RD template and relaxed using MD. Subsequently, the intramolecular interactions, filtered so as to catch those in the chains of highly conserved receptor residues, were analyzed. Although the intra-bundle interactions were tested and compared for both old- and new-type models of RD, V1aR, V2R and OTR, only sample interaction matrices for the new RD model are shown in Fig. 2, while a representative interaction net made by 26 conservative residues is presented in Fig. 3, again for the new RD model. Despite the characteristics of an inactive receptor, one may hope that this network may be typical of the whole RD-like GPCR family and pertinent to the general mechanism of signal transduction in this family of receptors.

Thus, a possible transduction scenario may involve initial interactions among TM6 Y268 (see in Fig. 3 the highest-sited member of the network, being in direct contact with the agonists) and its nearest-neighbors, TM6 W265, C264, F261, TM3 A124, TM7 S298 and A299, of which the latter two are simultaneously part of what we chose to term the "right switch". As such, they are located in the lower-right side in the projection in Fig. 3 and belong to the so called polar cluster, made of a set of conserved polar residues from TM1 N55, TM2 D83, TM7 S298, A299 (in most other GPCRs there are N and S, respectively, at the equivalent positions in TM7), N302 and Y306, near the cytosolic side of the receptor [13, 14]. Thus, we hypothesize that *via* this right switch the signal may be further transmitted to TM7 Y306, which, with an assistance of TM6 V254 (in most other GPCRs there is a T at the equivalent position) or directly, may talk with TM3 R135, belonging to the TM3 (D/E)RY conservative motif, central to the GPCR – G protein communication [13]. A complementary, more direct information flow *via* the "left switch" along TM6 and TM3 (TM5) conservative residues, see Fig. 3, seems possible although less likely.

As a side product, our analysis of interactions reveals also (not shown) that the new relaxed models are more optimally packed than the old ones, this being consistently reflected

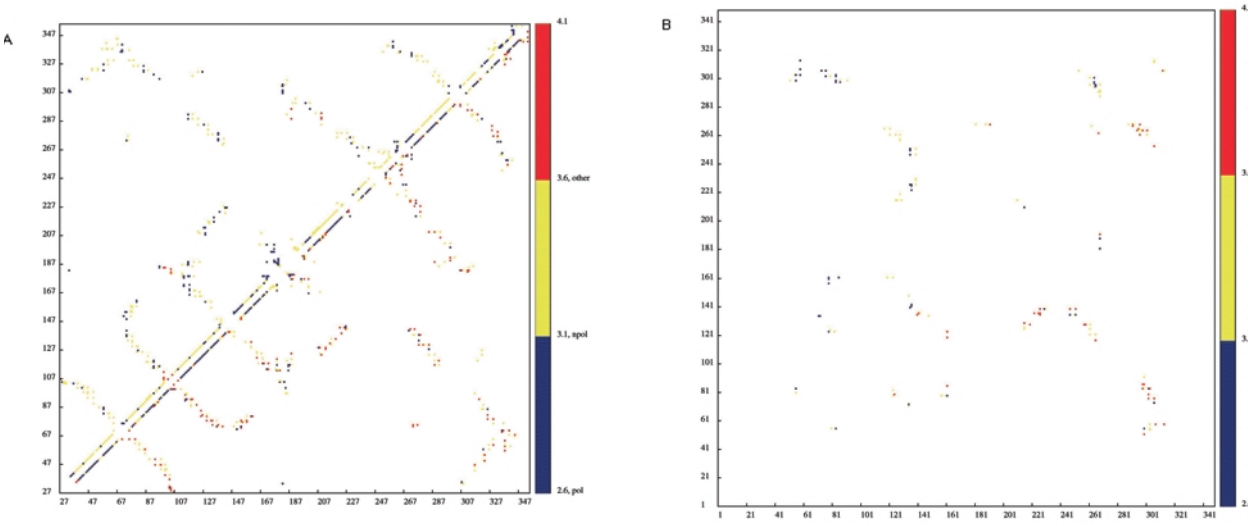

Figure 2. Residue–residue interaction map.

For clarity, the pairs of residues closer than six positions away in the sequence are neglected, as those making abundant nearest-neighbor and medium-range interactions, represented by (not shown) points along the diagonal. All remaining residues, any pair of atoms of which is within a distance of 2.6–4.1 Å are represented by colored dots: in the upper triangle according to polarity of the interactions: polar–polar (blue), nonpolar–nonpolar (yellow), other (red); in the lower triangle according to the distance: 2.6–3.1 Å (blue), 3.1–3.6 Å (yellow), 3.6–4.1 Å (red). **A**: The map for the relaxed experimental RD. The interacting parallel and antiparallel TM helices are represented by dotted traces parallel and perpendicular, respectively, to the diagonal. E.g., the consecutive interacting helices TM1–TM2, TM2–TM3 and so on, are represented by the 1st, 2nd and so on, respectively, dotted traces, consecutively crossing the diagonal from the lower-left corner on. Other inter-TM interactions sets can be traced accordingly. **B**: Same as **A**, but with all interactions not involving the conservative residues, see Table 1, filtered off.

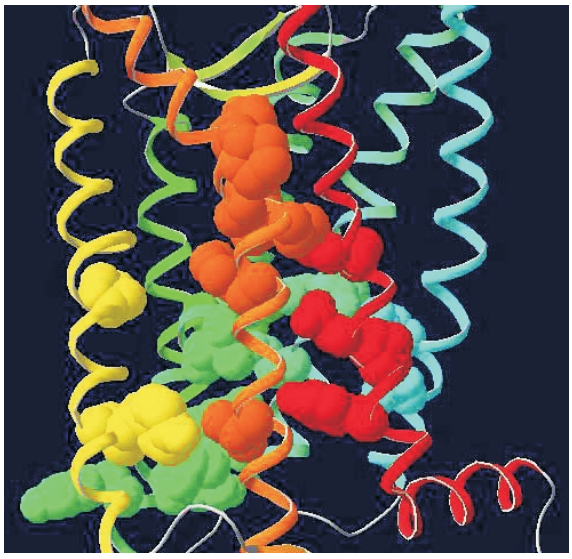

Figure 3. A network of generally conserved residues and their nearest neighbors potentially involved in signal transduction in the RD-like GPCR family.

The receptor orientation and coloring are the same as in Fig. 1. Ligand nest: TM6(F261, C264, W265, P267-on bundle exterior, Y268), TM3(A124); TM7-(S298, A299). Right switch: TM7(S298, A299, N302, P303), TM2(D83), TM1(N55, T58), TM7(Y306), TM6(V254). Left switch: TM6(F261), TM3(L128, L131), TM6(V254), TM3(E134, R135). Other noticeable inter-TM-helical snaps: TM1(N55)–TM2(D83); TM3(R135, Y136)–TM5(C222, Y223); and TM2(A82, N78)–TM4(W161).

in more extensive (by 20–35%) sets of intramolecular interactions in the former than in the latter, both in general and among the conserved residues.

We acknowledge Professor Henry I. Mosberg of the University of Michigan (Ann Arbor, U.S.A.) for making the G protein-coupled receptor templates available for public use.

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