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A common motif in G-protein-coupled seven transmembrane helix receptors

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SUMMARY

G-protein-coupled receptors all share the seven transmembrane helix motif similar to bacteriorhodopsin. This similarity was exploited to build models for these receptors. From an analysis of a multi-sequence alignment of 225 G-protein-coupled receptors belonging to the rhodopsin-like superfamily, conclusions could be drawn about functional residues. Seven residues in the transmembrane regions are conserved throughout all aligned receptors. These residues cluster at the cytosolic side of the transmembrane helices and are for all rhodopsin-like G-protein-coupled receptors implied in signal transduction. An analysis of correlated mutations reveals a number of residues, both in the helices and in the cytosolic loops, that might be important in the signal transduction pathway in subfamilies of this receptor family.

INTRODUCTION

G-protein-coupled receptors (GPCRs) constitute a large group of membrane-spanning receptors of diverse biological function. It is estimated that at least several hundred different members of these proteins exist [1–5]. The receptors have been divided into three superfamilies [6]:

(1) rhodopsin-like receptors,
(2) secretin-like receptors, and
(3) metabotropic glutamate receptors.

The rhodopsin-like receptors are characterized by the following conserved residues: GN in helix I, LxxxD in helix II, DRY at the end of helix III and NP in helix VII. Not enough secretin-like and metabotropic glutamate sequences have been determined yet to define sequence conservation patterns in these subfamilies. The secretin-like and metabotropic glutamate receptors that have been sequenced so far do not possess any of the residues characteristic for the GPCR family [7,8].

Some important GPCRs of the rhodopsin-like group are amine receptors (α- and β-adrenergic,

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muscarinic, serotonin, dopamine and histamine), peptide receptors (angiotensin II, bradykinin, endothelins, tachykinins, neuropeptides, chemotactic peptides), interleukin-8, olfactory and tropin receptors, and opsins. Their activation leads to varied basic physiological responses, but can also induce complex behavioral patterns such as affection, depression, aggression, etc.

The basic function for all GPCRs seems to be similar. An external signal is received, which is normally a ligand but in the case of opsins a photon alters the structure of the bound retinal. This external signal is transduced through the membrane to the cytosolic side where a G-protein is activated. The signal reception and transduction is performed mostly by the membrane-embedded part of the receptors. The specificity for G-proteins is most likely determined by the cytosolic loops [9].

No direct 3D structure determination of any GPCR has been carried out yet. However, the structure of the membrane-embedded helices of bacteriorhodopsin (Brh) has been solved by electron crystallography [10]. Experimental data from deletion mutations, antibody targeting and proteolytic digestion experiments provide evidence that the overall features of GPCRs and Brh must be similar. Brh has seven transmembrane helices organized in a kidney-shaped manner, with nearest-neighbor helices running antiparallel. It has been determined experimentally that GPCRs also possess seven transmembrane helices (for reviews see Refs. 11,12 and references cited therein). Many attempts have been made to derive three-dimensional models of GPCRs based on this similarity with Brh [13–15]. This 3D modeling has shown that point mutations and inhibitor binding can be in agreement with the assumption that the 7TMs of GPCRs and Brh are similar.

Model building has to start with a sequence alignment between the GPCRs and Brh. A problem is that standard sequence alignment techniques use standard amino acid exchange matrices. These matrices have been optimized for the alignment of water-soluble proteins and are far from ideal for membrane-embedded proteins. Another problem is that the overall sequence identity between 7TMs and Brh is too low to allow for model building by homology using standard techniques [16].

We used a novel iterative profile alignment method that is based on knowledge about the function of some key residues to align 225 rhodopsin-like GPCRs. Many additional conserved residues were observed and, together with an analysis of correlated mutations, a series of residues potentially important for signal transduction was detected. These residues are located mainly in the helices, but ten of the residues that display correlated mutational behavior were found to lie in the cytosolic loops.

ITERATIVE SEQUENCE ALIGNMENT

Many attempts have been made to align GPCR sequences [e.g., 4,13,14,17–25]. Standard sequence alignment techniques can be used reliably when only (highly homologous) sequences within one class of receptors are aligned, but fail when all presently known receptors need to be aligned. The problem is the occurrence of insertions and deletions (indels), which of course are very unlikely in the transmembrane helices.

The work of Cronet et al. [15] made clear that general rules for residue exchanges in 7TMs are hard to derive because residue preferences are very position specific. A good Dayhoff-type matrix can therefore not be derived for the transmembrane helices. Since we have a relatively large

number of sequences to work with (225), we chose to use a profile alignment method [26,27] utilizing a unitary exchange matrix, or in other words, to set the profile values equal to the frequency of occurrence of the 20 amino acids at each position in the multi-sequence alignment.

The initial alignment of the 7TM sequences of ~100 known GPCRs, extracted from the SWISS-PROT [28] and PIR [29] databases, was made by visual inspection. In this alignment, the very large insertions that often occur between helices V and VI were left out. The profile obtained from the initial manual alignment was used to realign the sequences. This process of rebuilding the profile from the multi-sequence alignment and aligning all sequences one by one against the profile was iterated until no changes occurred between two subsequent alignments. The gap-open penalty was kept at six times the maximal profile value for residues known from biochemical experiments to lie in the transmembrane helices and at one time the maximal profile value elsewhere. This completely prevented indels from occurring in helices. The gap elongation penalty was 0.1 times the maximal profile value. To accommodate the large differences in loop lengths, the six residues in the middle of the loops between the helices were given a gap elongation penalty of 0.0. The program MAXHOM [30] was used for alignments and database searches.

When the SWISS-PROT database [28] was screened with this profile, all rhodopsin-like receptors but no secretin-like or glutamate receptors were extracted, and zero false positives appeared. When the acceptance threshold was lowered such that other proteins were also detected, most of these proteins were GPCRs of other groups and ion channels. To check that our profile was a generalized description of rhodopsin-like GPCRs and not merely a description of the sequences

TABLE 1
LOCATION OF LIGAND BINDING RESIDUES IN THE 7TM STRUCTURE[a]

Helix II

Asp^{383} (224) in lutropin receptor is important for hormone binding and G-protein activation [29].
Asp^{79} (224) is related to sodium regulation of α2-adrenergic receptors [47].
Ser^{120} (231) is involved in antagonist binding in muscarinic receptors [48].

Helix III

$Asp^{(323)}$ neutralizes the positive charge of the ligands in amine receptors [49]. This residue corresponds to Asp^{105} in the human M1 muscarinic receptor; to Asp^{113} in the rat β-2 adrenergic receptor; and to Asp^{98} in the rat histamine H2 receptor. $Phe^{(320)}$, $Ala^{(322)}$, $Trp^{(331)}$ and $Ser^{(332)}$ (corresponding to Phe^{115}, Ala^{117}, Trp^{126} and Ser^{127} in bovine rhodopsin) are proximal to the retinal [50–52]. Glu^{122} in bovine rhodopsin (327) is the counterion for the Schiff base [53–56].

Helix V

$Ser^{(513)}$ and $Ser^{(516)}$ (corresponding to Ser^{204} and Ser^{208} in the β-2 adrenergic receptor [57]) hydrogen bond with ligand hydroxyl groups. Thr^{231} (509) and Thr^{234} (512) bind agonists in the muscarinic M1 receptor [48]. Asp^{185} (512) and Thr^{189} (516) form a serine proteinase-like catalytic relay with the imidazole ring of the ligand in the rat histamine H2 receptor [58,59]. Asn^{200} (509) in the rat bradykinin B2 receptor is thought to be associated with ligand binding [60]. His^{211} (516) in bovine rhodopsin modulates the mechanism of deprotonation of the Schiff base [61].

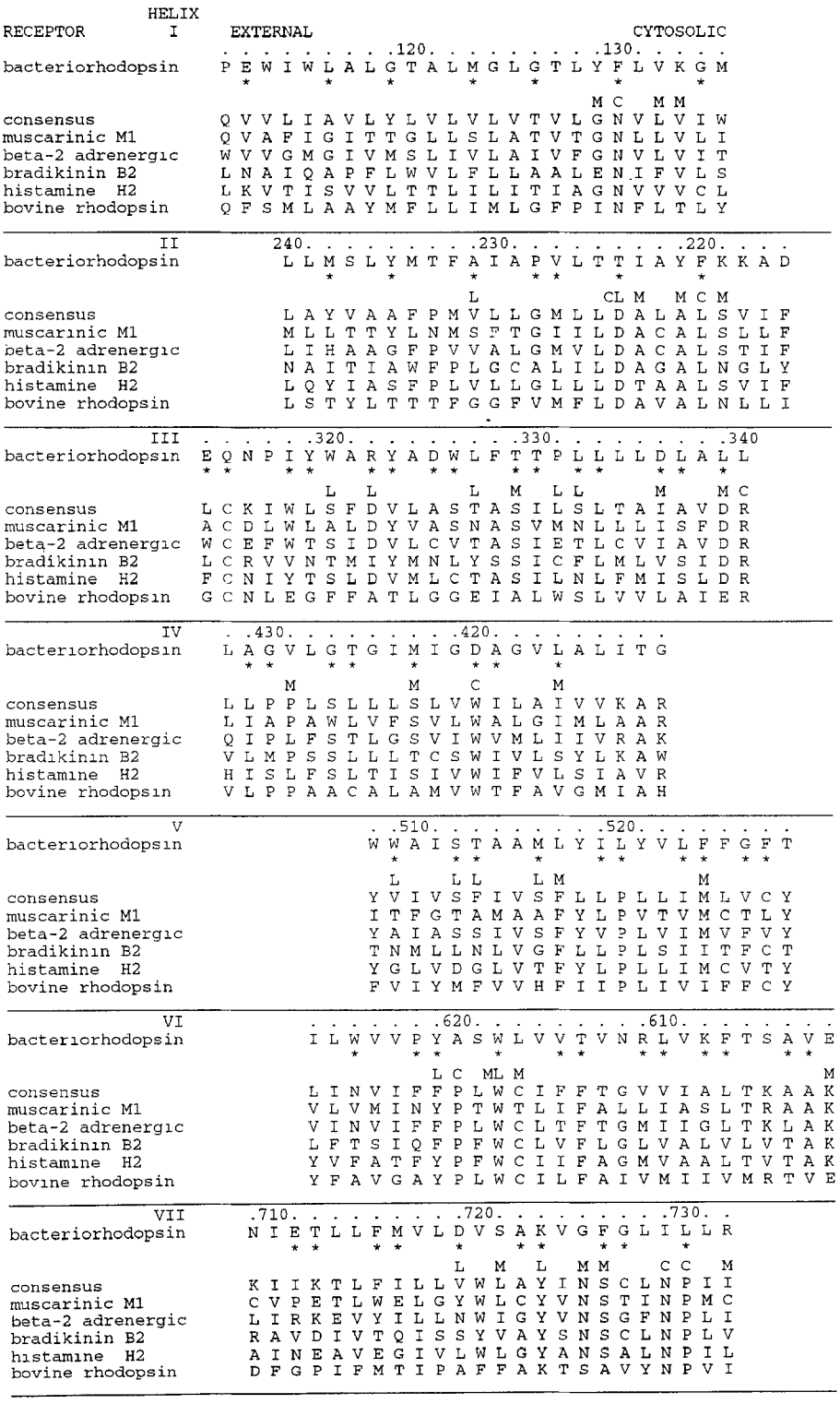
Helix VI

Phe^{290} (621) in the β-2 adrenergic receptor is thought to bind the ligand [62]. Tyr^{506} (621) in the muscarinic receptors are thought to bind the ligand [48]. Trp^{265} (618) and Tyr^{268} (621) are close to the retinal in bovine rhodopsin [52].

Helix VII

Lys^{296} (723) binds the retinal in bovine rhodopsin [63–66]. Tyr^{529} (719) and Tyr^{533} (723) are putatively related to ligand binding in muscarinic receptors [48].

[a] Numbers are from the original sequence. Numbers in parentheses refer to the numbering of Fig. 1.


HELIX
I
RECEPTOR
EXTERNAL
CYTOSOLIC
bacteriorhodopsin
consensus
muscarinic M1
beta-2 adrenergic
bradikinin B2
histamine H2
bovine rhodopsin
II
III
IV
V
VI
VII

used, a jack-knife experiment was performed. A profile was constructed as described above based on ~125 receptors; all opsins and olfactory receptors were excluded. When this profile was used to screen the SWISS-PROT database, exactly the same sequences were detected as mentioned before. Several such jack-knife experiments were performed, always with the same result.

The sequence alignment of the 7TMs of these GPCRs is shown in Fig. 1. For a limited set of GPCRs experimental data about residues involved in ligand binding exist (see Table 1); these residues are indicated in Fig. 1.

CORRELATED MUTATIONS

In the multi-sequence alignment, several classes of residues can be identified (see also Table 1): (1) Ligand binding residues. These are mostly very variable, but some are conserved in only one class of receptors (e.g., the aspartic acid that binds to the positive charge in all amine receptors); (2) Highly conserved residues. For several of these a role in signal transduction has been observed experimentally (see Table 1); (3) Residues that are conserved in subfamilies but differ between subfamilies. These residues have correlated mutational behavior when the total multi-sequence alignment is analyzed. We used a method similar to the one described by Goebel et al. [31] to find pairs of residues that show a correlated pattern of conservation and mutation. Pairs of residues that are both 40–90% conserved, but which display simultaneous mutation to the same residue in more than 67% of all cases, were determined. These residues determine the principal functional differences between sequence subfamilies [see e.g. Refs. 32,33]. Forty-three residue pairs were found and analyzed. Surprisingly, 42 of these 43 pairs form a network of residues connected by correlated mutational behavior (see Fig. 2). Ten of these residues are located just outside the helices in the cytosolic loops.

3D MODEL BUILDING

The alignment of G-protein-coupled receptor 7TMs with Brh is not straightforward. Different techniques have been tried to solve this problem [e.g. Refs. 14,15]. We combined the alignment of the β-2 adrenergic receptor with Brh by Cronet et al. [15], and the alignment of five receptor classes with Brh by Trumpp-Kallmeyer et al. [14], but made some minor modifications to make a larger number of highly conserved helical residues point away from the lipid. The rationale for this is that there is virtually no evolutionary pressure on the residues that point towards the lipid

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Fig. 1. Spatially aligned sequences of Brh and some representative GPCRs. The sequences of the seven helices of the G-protein-coupled receptors mentioned in Table 1 are displayed in their putative alignment with Brh. Top: sequence of the 7TM of Brh. Asterisks indicate residues that point into the central cavity. Note the reverse order of helices II, IV and VI. The consensus sequence was taken from the multi-sequence alignment. Even-numbered sequences run from right to left, thus the extracellular side of the helices is at the left and the cytosolic side at the right. Residues that align vertically in this figure are equally deep in the membrane in the model shown in Fig. 2. L, C, or M above the residues indicate Ligand binding, Conserved, or correlated Mutation residue, respectively. The residues are numbered similarly as described by Hibert et al. [13,14]: residues are numbered such that the first digit indicates the helix number. In each helix the number of the most conserved residue is a multiple of 10 while the start of the helix is at the same time as close as possible to 100 times the number of the helix.

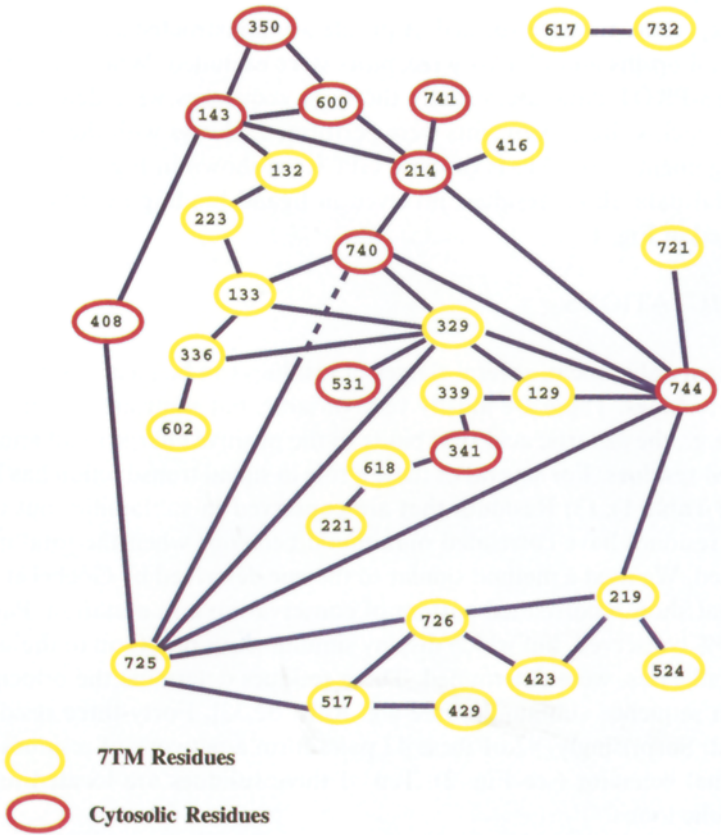

Fig. 2. Correlated mutations. Residues with pairwise correlated mutational behavior are connected. Numbering as in Fig. 1.

bilayer, except that they should preferably be hydrophobic, so no residues facing the lipid are expected to be highly conserved.

The final correspondence between the 7TM sequences of the GPCRs and the Brh structural framework is shown in Fig. 1. In this figure, the residues known to point inwards in Brh and the residues falling in one of the three aforementioned classes (conserved, correlated mutating, ligand binding) are indicated. It can be seen that most of these residues correspond to positions in Brh that point inwards. Figure 3 schematically shows the average putative G-protein-coupled 7TM structure with the three types of residues color coded. The models of course are incorrect in their details, but they have proven useful in enlarging our understanding of the functioning of the GPCRs [e.g. Refs. 13,14,34].

DISCUSSION

The function of GPCRs is to detect an external signal and convert this to an action at the cytosolic side of the 7TM that leads to G-protein activation. In order to perform such a role, these receptors first have to interact with a specific ligand (light absorption for opsins) at the periplas-

Fig. 3. Location of residues in the 7TM model. Cylinders run through the model of the average 7TM. The vertical location of the three classes of residues (ligand binding (green), conserved (blue), or correlated mutating (red)) are indicated.

mic side of the membrane. This ligand binding should result in a signal that is sent through the receptor, resulting in activation of the G-protein at the cytosolic side of the membrane.

The alignment of the GPCR sequences revealed that the residues known to be involved in ligand binding (Table 1) are mainly found in the middle and in the extracellular half of the 7TM, whereas a large number of highly conserved residues are found in the cytosolic half (Fig. 3). In globular proteins, a very high level of sequence conservation is almost always associated with an important role in function (e.g., the conservation of a serine in serine-proteinases or a histidine in haemoglobins) rather than with the maintenance of structure. It therefore seems likely that the sequence conservation in the 7TMs of the GPCRs is related to a common function of all these receptors, namely transducing a signal through the membrane.

Several residue positions in GPCRs have correlated mutational behavior. These residues are mostly located at the cytosolic half of the 7TMs and in the intracellular loops and show correlated mutational behavior mainly between the GPCR subfamilies, i.e., they are conserved if only one subfamily is analyzed. The fact that these residues are highly conserved within every subfamily strongly suggests that they have a functional role in those subfamilies. Their close spatial proximity to residues known to be involved in signal transduction suggests that this function is signal transduction too. The fact that they are often different between subfamilies indicates that they are involved in the receptor-specific part of the signal transduction pathway.

Two facts corroborate this hypothesis: (1) Three residues that are part of the large correlated mutation network shown in Fig. 2 lie in loops that are known to be necessary for binding and activation of G-proteins. One residue (Glu^{600}) is located next to a peptide that is crucial for G-protein activation in rhodopsin [35,36], the α-adrenergic [37–39], the β-adrenergic [40–43], and the muscarinic [44] receptors. The other two residues are phenylalanines, located in the middle of

a stretch of residues immediately after helix VII in the C-terminal domain, which is known to be necessary for binding and subsequent activation of several G-proteins [9,37]; (2) The sequence motif DRY at the cytosolic end of helix III has been shown important for G-protein binding [36,45,46]. Two of these three residues (D and Y) are part of the network of correlated mutations.

Many questions remain about GPCRs. Not much is known about the functional role of extracellular loops, and the details of signal transduction are far from being understood. The mode of interaction of more sophisticated ligands such as peptides and proteins with GPCRs and the interaction of the receptors with G-proteins remain to be elucidated. Three-dimensional models of GPCRs could be used to better understand their structure–function relationship. Powerful information for modeling would be provided if mutational studies could prove that the highly conserved residues and the residue pairs with correlated mutational behavior are indeed involved in signal transduction.

The more reliable receptor structure models that result will be of great benefit for the rational design of new medicines. The multi-sequence alignment of 225 G-protein-coupled receptors has been deposited in the TM7 file server. Send HELP to 'TM7@EMBL-Heidelberg.DE' to obtain information about GPCR-related data and topics, or send, to this same internet address, the message GET ALIGN:ALL.ALIGN.

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