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An analysis of the periodicity of conserved residues in sequence alignments of G-protein coupled receptors

Implications for the three-dimensional structure

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Twenty-three sequences from the family of G-protein coupled receptors have been aligned according to the 'historical alignment' procedure of Feng and Doolittle. Fourier transform analysis of this reveals that parts of five of the seven putative membrane-spanning regions exhibit a periodicity of conserved/nonconserved residues which is compatible with the periodicity of the α-helix. This would place the conserved residues on one side of the helix, which may face the inside of the proposed seven membered helical bundle.

Primary structure; Sequence alignment; Membrane topology; Membrane-spanning domain

1. INTRODUCTION

Receptors linked to second messenger production through guanine nucleotide-binding regulatory proteins (G-proteins) are fast becoming one of the most important receptor systems discovered to date. The incredible diversity of the ligands which bind to these receptors and the equally diverse nature of the cellular effects of this binding are surprisingly complemented by a high degree of sequence homology in the receptors themselves, especially in the seven proposed hydrophobic regions ([1] for review).

Secondary structure predictions and hydrophobicity and hydrophobic moment plots predict seven transmembrane helices and give similar results for the different classes of these receptors suggesting a similar topology throughout the family [2-6]. The proposed transmembrane topologies of these proteins consist of a helical bundle containing seven transmembrane helices arranged in a similar fashion to the low-resolution electron density map of bacteriorhodopsin [7]. Spectral evidence [8-12] suggests that this comparison is justified despite the lack of sequence homology between the prokaryotic and eukaryotic proteins. Protease protection and chemical probe experiments have determined the location of individual residues relative to the membrane [13-24]. Information on the disposition of amino acid residues in the transmembrane segments was obtained using a photoactivated nitrene-generating hydrophobic probe which identified 20 nucleophilic sidechains of ovine rhodopsin exposed to the lipid domain [25,26].

X-ray analysis of crystals of the reaction centre from *Rhodopseudomonas viridis* allowed the calculation of an electron-density map at 3 Å resolution [27,28]. The structure of the *Rhodobacter sphaeroides* reaction centre was later determined to a resolution of 2.8 Å [29-33]. This system represents the only high-resolution structure of an integral membrane protein. Sequence alignments and Fourier transform analysis of the periodicity of conserved/nonconserved redisues have recently

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been described for the reaction centre [34]. This analysis shows that the conserved residues in certain helices exhibit a periodicity which is compatible with them being on one side of an α-helix. These helices are on the surface of the reaction centre. Analysis of integral membrane proteins has shown that surface residues are poorly conserved [31]. Hence helices which are on the surface of proteins should have a conserved internal facing side and a variable external side (facing the lipid) and we refer to these helices as amphipathic.

We propose a model for the G-protein coupled receptors based solely on the multiple alignment of the sequences from 23 receptors in this class. The method is based on the analysis of the periodicity of conserved residues in the putative transmembrane regions following the principle outlined above [34]. The proposed seven membered helical bundle [1,7] of these proteins provides the possibility that all seven helices may have a conserved internal face and a variable external surface. This method may thus be useful in the modelling of these receptors.

2. METHODS

Sequence alignments were carried out on 23 different sequences of G-protein coupled receptors. The multiple alignment of the sequences was obtained with the 'historical alignment' procedure of Feng and Doolittle [35]. In this procedure sequences are incorporated into the multiple alignment according to the topology of an initial phylogenetic tree constructed on the basis of pairwise alignments and the distance metric of Feng et al. [36].

The sequences used [37–53] were the muscarinic cholinergic receptors (human subtypes M1, M2, M3, M4, M5, rat subtypes M1, M2, M3, M4, M5, and porcine subtypes M1, M2), α-adrenergic receptor (human kidney), β-adrenergic receptors (human β1 and β2, avian β1, hamster β2), 5HT-1A receptor (human), 5HT-1C receptor (rat), 5HT-2 receptor (rat), the D2 dopamine receptor (rat), bovine substance K receptor and bovine rhodopsin.

The extent of the helical regions in this analysis (fig.6) was based on the model of ovine rhodopsin [7] and on hydrophobicity studies, with the condition that the 23 sequences used aligned well in these regions. Single residue deletions and insertions in the alignment which occurred in these helical regions were adjusted so that an equal number of sequential residues was compared. This is necessary if regular helices are to be presumed. Gaps and insertions occurred in the centre of helix 2 and helix 4, at the N-terminus of helix 1 and at the C-terminus of helix 4 and helix 6. There were no occurrences of insertions or deletions of more than one residue.

Fourier transform analysis was carried out on these regions as previously described for the reaction centre [34]. The power spectra $P(\omega)$ may be defined as

$$P(\omega) = \left[\sum_{j=1}^{N} (V_j - \bar{V}_j)\cos(j\omega)\right]^2 + \left[\sum_{j=1}^{N} (V_j - \bar{V}_j)\sin(j\omega)\right]^2$$

where V_j, the number of different amino acids at each position in the sequence alignment; $\bar{V}_j$, the mean value of V_j; ω, the rotation angle between adjacent residues in the helix; j, the position of the amino acids in the helix. The plot of $P(\omega)$ against ω gives a value of ω_{max} at about 100°.

The number of residues preferentially conserved on one side of the helix can be characterized by ψ where

$$\psi = \left[1/30° \int_{90°}^{120°} P(\omega)\mathrm{d}\omega\right] \Big/ \left[1/180° \int_{0°}^{180°} P(\omega)\mathrm{d}\omega\right]$$

and represents the average value of $P(\omega)$ in the α-helical region (ω = 90–120°). A value of greater than 2 was taken as a sign of an amphiphatic helix [34].

The conservancy at each position can also be defined as C where $C = z/V_j$ (z = the number of sequences used in the alignment). C can be plotted as a helical wheel to show pictorially the amphipathic nature of the helix (fig.3a). The value of ω_{max} is used in this plot to show the maximum periodicity so long as it is compatible with an α-helical conformation, that is between 3.4 and 3.6 residues per turn. The heavy arrow represents a moment of conservancy and is the sum of the individual vectors calculated from $C(\omega)$ where

$$C(\omega) = \left[\sum_{j=1}^{N} C\cos(j\omega)\right]^2 + \left[\sum_{j=1}^{N} C\sin(j\omega)\right]^2$$

The helical plot however does not show at which vertical points in the putative helix the conservancy occurs. This can be seen by plotting the most conserved 160° of the helical wheel on one side of a 'helical rod' (fig.3b) and the remaining variable region on the opposite side. The actual length of the amphipathic regions in the putative helix can then be appreciated and $P(\omega)$, $C(\omega)$ and ψ can be recalculated for each of these amphipathic regions.

3. RESULTS AND DISCUSSION

Fig.1a shows the alignment of the first hydrophobic region with all insertions and deletions removed and fig.1b shows the variance across this helical region. The Fourier transform of this (fig.2) shows a peak at about 105° which corresponds to a periodicity of approximately 3.4 residues per turn which is compatible with an α-helical conformation.

The surface residues of globular and integral membrane proteins are less conserved than those buried in the centre. A peripheral α-helix is exposed to both the inside and the outside of a protein and hence we would expect a periodicity of conservancy corresponding to the number of residues per turn of the helix. The normalized area of the peak, ψ, provides a measure of the exposure of the helix of

a

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MR HM1   Q V A F I G I T T G L L S L A T V T G N L L V L I S F K V N T E L
PIGM1    Q V A F I G I T T G L L S L A T V T G N L L V L I S F K V N T E L
RAT M1   Q V A F I G I T T G L L S L A T V T G N L L V L I S F K V N T E L
MR HM3   Q V V F I A F L T G I L A L V T I I G N I L V I V S F K V N K Q L
RAT M3   Q V V F I A F L T G F L A L V T I I G N I L V I V A F K V N K Q L
PIGM2    E V V F I V L V A G S L S L V T I I G N I L V M V S I K V N R H L
RAT M2   E V V F I V L V A G S L S L V T I I G N I L V M V S I K V S R H L
MR HM2   E V V F I V L V A G S L S L V T I I G N I L V M V S I K V N R H L
MR HM4   E M V F I A T V T G S L S L V T V V G N I L V M L S I K V N R Q L
RAT M4   E M V F I A T V T G S L S L V T V V G N I L V M L S I K V N R Q L
RAT M5   E V I T I A V V T A V V S L M T I V G N V L V M I S F K V N S Q L
MR HM5   E V I T I A A V T A V V S L I T I V G N V L V M I S F K V N S Q K
BARAVI   W E A G M S L L M A L V V L L I V A G N V L V I A A I G R T Q R L
B2HUM    W V V G M G I V M S L I V L A I V F G N V L V I T A I A K F E R L
B2HAM    W V V G M A I L M S V I V L A I V F G N V L V I T A I A K F E R L
B1HUM    W T A G M G L L M A L I V L L I V A G N V L V I V A I A K T P R L
HA2 AR   A V A G L A A V V G F L I V F T V V G N V L V V I A V L T S R A L
5HT-2    E K N W S A L L T T V V I I L T I A G N I L V I M A V S L E K K L
5HT-1A   Y Q V I T S L L L G T L I F C A V L G N A C V V A A I A L E R S L
D2-DOP   H Y N Y Y A M L L T L L I F I I V F G N V L V C M A V S R E K A L
5HT-1C   V Q N W P A L S I V V I I I M T I G G N I L V I M A V S M E K K L
SKRBOV   Q L A L W T A A Y L A L V L V A V M G N A T V I W I I L A H Q R M
RHBOV    Q F S M L A A Y M F L L I M L G F P I N F L T L Y V T V Q H K K L

Vj=      8 10 6 8 8 5 7 6 7 7 7 3 4 5 7 4 3 9 2 1 5 3 2 5 8 4 4 6 8 6 7 8 2
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b

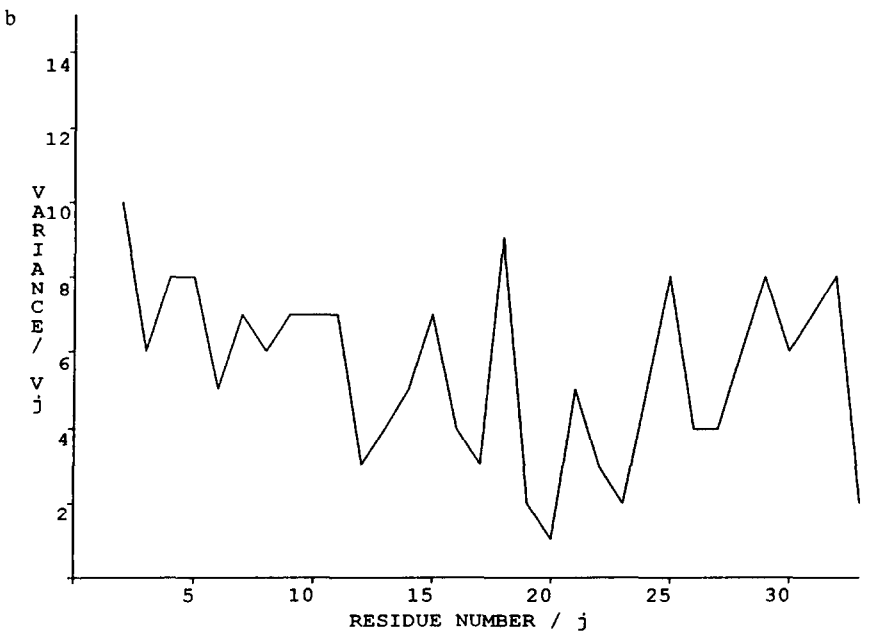

Fig.1. (a) Alignment for the sequences corresponding to helix 1. V_j corresponds to the number of different residue types at each position. The conserved asparagine is indicated with an underscore. The sequences used are the muscarinic cholinergic receptors (human subtypes, MR HM1 – MR HM5, rat subtypes RAT M1 – RAT M5, and porcine subtypes PIG M1 and PIG M2), human $\alpha 2$ adrenergic receptor (HA2AR), β adrenergic receptors (human subtypes B1 HUM and B2 HUM, avian BAR AV1, hamster B2 HAM), 5HT-1A receptor (human), 5HT-1C receptor (rat), 5HT-2 receptor (rat), the D2 dopamine receptor (rat), bovine substance K receptor (SKRBOV) and bovine rhodopsin (RHBOV). (b) The plot of V_j against residue number (j) for helix 1 shows how the conservancy varies over the putative transmembrane region.

an integral membrane protein to the membrane as previously described for the reaction centre. The calculated values of ψ for helices A and B of the reaction centre are 2.5 for each helix. The values for the seven helices of the G-protein coupled receptors are summarised in table 1.

The helical plot of conservancy for helix 1 (fig.3a) shows pictorially the amphipathic nature of the helix. The vertical plot (fig.3b) shows that the C-terminal end of the first helix is much more amphipathic than the N-terminal end. The Fourier transform of the most amphipathic section of each helix (16 residues) (fig.4) results in a value of ψ_{16} greater than 2 for six of the seven helical regions (table 1) suggesting that the amphipathicity is more pronounced in these sections of helix.

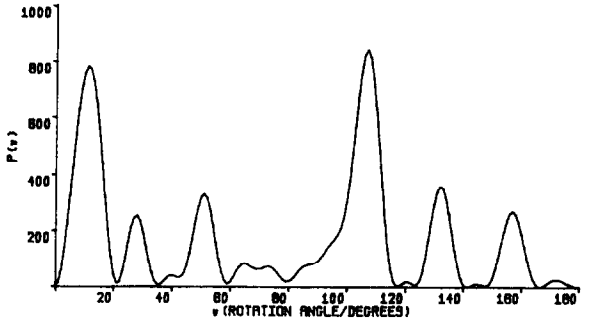

Fig.2. The Fourier transform spectrum $P(\omega)$ for the first transmembrane region (33 residues). The peak at 105° corresponds to a periodicity of about 3.4 residues per turn. The normalised area of the peak ψ gives a value of 2.2, which is indicative of an amphipathic helix.

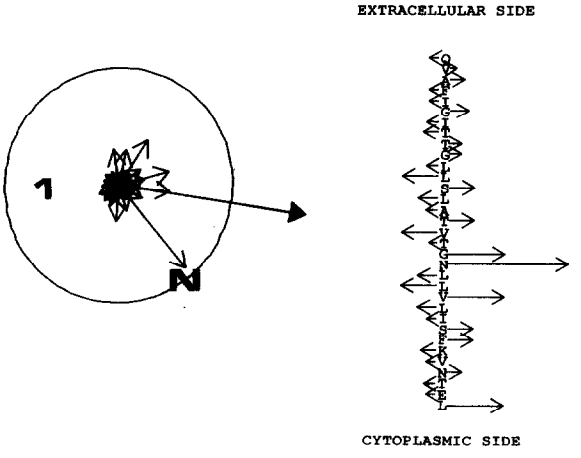

Fig.3. (a) The helical wheel depiction of the amphipathicity of helix 1. The conservancy C is taken as z/V_j where z is the number of sequences used in the analysis. The heavy line is the sum of the individual vectors and points to the interior of the helical bundle. The rotation angle (ω) is ω_{max} from the Fourier transform (fig.2) and in this case is about 105°. (b) The vertical plot representation of the amphipathicity of helix 1. The sequence given refers to the M1 subtype of the human muscarinic cholinergic receptor. The right side of the plot represents the most conserved 160° of the helical wheel plot (depicted in a). It clearly shows that the amphipathicity of helix 1 is confined to the carboxy-terminal region of the putative membrane spanning region, that is the cytoplasmic end.

Although the value of ψ_{16} of helix 7 is 3.1, the Fourier transform plot of this region (not shown) shows a broad peak at 84° and another at 114° which result in this high value of ψ_{16}, even though there is actually a trough at 100°. The helical wheel plot of this helix using 3.4 residues per turn (fig.5) does however show that the helix has a more conserved side. However the vertical plot (fig.6) shows that the sequence corresponding to L-C-Y(407)-V-N-S-T-I does not show amphipathic character. Coupled with the unusual Fourier transform plot, this may reflect the presence of the distorted region predicted previously in rhodopsins in the region of the retinal binding domain [2,3,7]. The equivalent position to the retinal binding lysine of bovine opsin in our alignment is the Y-407 in the human M1 subtype of the muscarinic cholinergic receptor.

The Fourier transform plot of helix 2 shows a broad peak over the helical region and does not

Table 1

Values of ψ calculated for the 7 helices of the G-protein complex receptors

Helix	No. of residues per helix	ψ	ψ_{16}	Residues per turn used in the helical wheels
1	33	2.2	2.6	3.4
2	29	1.1	1.9	3.4
3	32	2.1	3.6	3.6
4	26	2.7	3.5	3.4
5	33	2.4	2.7	3.6
6	34	1.7	2.7	3.6
7	22	1.9	3.1	3.4

The number of residues per turn used in the helical wheels was taken from ω_{max} between 100° and 106°. ψ reflects the preferential conservation of residues on one side of the helix. ψ_{16} refers to ψ calculated over a 16 residue length of sequence which shows the most amphipathicity. Values of ψ and ψ_{16} which are greater than 2 reflect a high probability of an amphipathic helix over the length of the sequence used

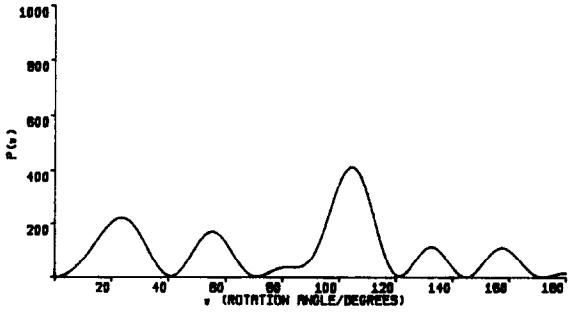

Fig.4. The Fourier transform of the 16 residues at the carboxy-terminal end of helix 1 shows a similar peak at 105°. The value of ψ_{16} in this case is 2.6 showing a greater degree of amphipathicity in this region of the first membrane spanning region.

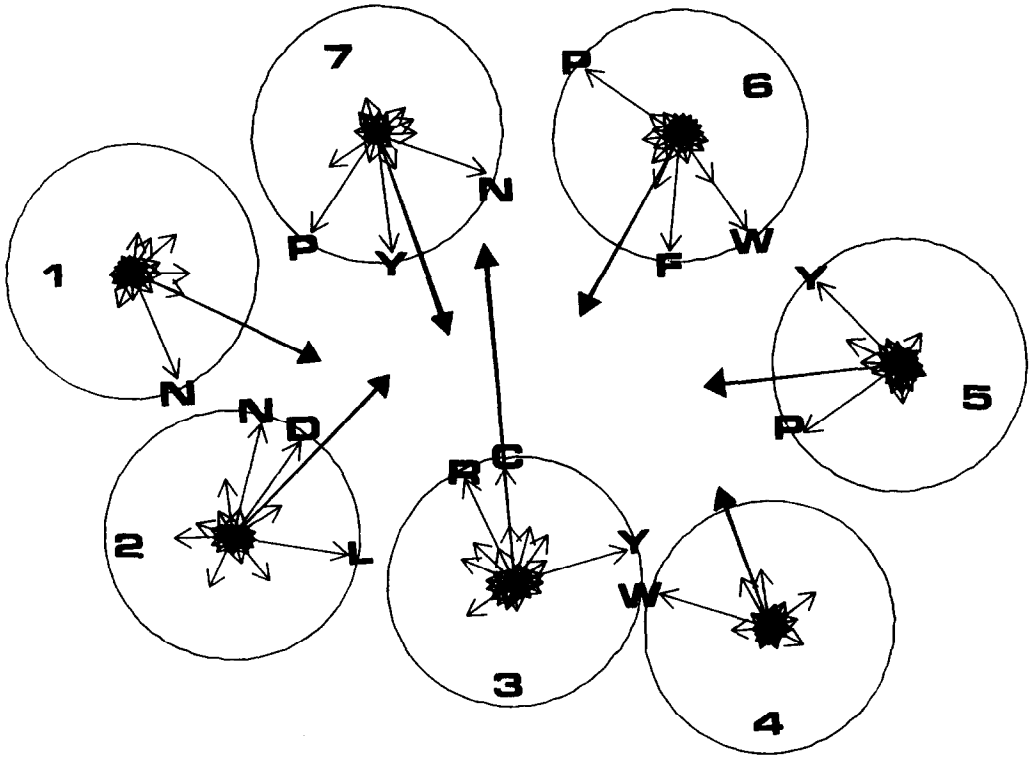

Fig.5. The helical wheel depiction of all seven helical regions arranged as if viewed from the extracellular side of the membrane. The helical bundle is arranged according to the seven high density regions of the electron density map of bacteriorhodopsin as described previously [7]. The membrane spanning regions are depicted so that the conserved side of each helix points towards the centre of the helical bundle.

show any convincing periodicity. This may have implications for its three-dimensional structure suggesting that this region is either not a regular helix or that it is not a peripheral helix.

The Fourier transform spectra for the sixteen residue sections of helices 1, 3, 4, 5 and 6 show a strong periodicity which is compatible with an α-helical conformation. We propose that the conserved side of these helices faces the interior of the helical bundle (fig.5) and that these regions have a regular helical structure. Since a helix of at least 20–22 residues is required to cross the membrane we suggest that the 16 residue sections which constitute the more amphipathic regions of the membrane spanning helices reside at a similar depth in the membrane (fig.6) and constitute a conserved core which is involved in helix packing and ligand binding.

This model can be compared with more experimental results on the disposition of amino acid residues in the transmembrane regions. Studies on the β-adrenergic receptor have revealed that two aspartate residues were critically important in ligand binding [55,56]. The equivalent residues in the human M1 muscarinic cholinergic subtype are D-71 and D-105 which reside in helices two and three, respectively. Our model places these residues on the inside of the helical bundle where the ligand is believed to bind. A third aspartate residue, D-122 on the cytoplasmic side of the third putative helix, may also be involved in ligand binding. However we find that this aspartate lies 120° from the vector sum of conserved residues. The sequence alignment of the third putative helical region is shown in fig.7.

The equivalent residue to the retinal binding lysine of bovine opsin is Y-407 and this too is found to reside on the inside of the helical bundle though as mentioned above, this region may not have a regular helical conformation.

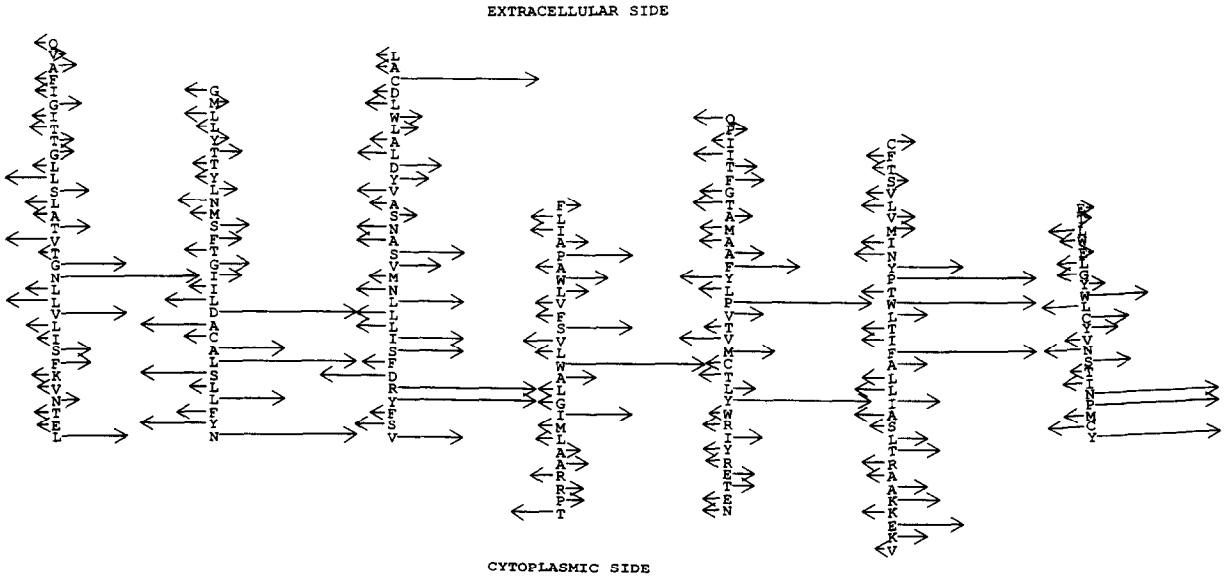

Fig.6. The vertical plots of all seven predicted membrane spanning regions arranged in an anti-parallel fashion as is believed to be the case in the helical bundle. The cytoplasmic side of the helical bundle is represented on the bottom of the diagram and the extracellular side on the top. The conserved side of the helix is depicted on the right hand side of the sequence of the human muscarinic cholinergic receptor subtype M1.

Other experimental studies on the disposition of amino acid residues in the helices include experiments in which a photoactivated nitrene-generating probe was used to selectively label residues on the outside of the helical bundle [7,25,26]. Our analysis is 60–65% compatible with these results. By definition, our method places the conserved residues, such as the conserved tryptophans in helices 4 and 6 and the conserved tyrosine of helix 5, on the inside of the helical bundle despite evidence that they face the lipid environment [7,25,26]. Any conserved residues on the outside of a surface helix will disrupt this approach since it undermines the initial premise that conserved residues reside on the inside. Placement of these three aromatic residues on the outside in our model (fig.5) would result in the movement of the proline residues in all three of these helices towards the inner face. These three proline residues, which lie at a similar depth in our model (fig.6), have been

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MR_HM1   D L W L A L D Y V A S N A S V M N L L L I S F D R_Y_F S V
PIGM1    D L W L A L D Y V A S N A S V M N L L L I S F D R_Y_F S V
RAT_M1   D L W L A L D Y V A S N A S V M N L L L I S F D R_Y_F S V
MR_HM3   D L W L A I D Y V A S N A S V M N L L V I S F D R_Y_F S I
RAT_M3   D L W L S I D Y V A A N A S V M N L L V I S F D R_Y_F S I
PIGM2    D L W L A L D Y V V S N A S V M N L L I I S F D R_Y_F C V
RAT_M2   D L W L A L D Y V V S N A S V M N L L I I S F D R_Y_F C V
MR_HM2   D L W L A L D Y V V S N A S V M N L L I I S F D R_Y_F C V
MR_HM4   D L W L A L D Y V V S N A S V M N L L I I S F D R_Y_F C V
RAT_M4   D L W L A L D Y V V S N A S V M N L L I I S F D R_Y_F C V
RAT_M5   D L W L A L D Y V A S N A S V M N L L V I S F D R_Y_F S I
MR_HM5   D L W L A L D Y V A S N A S V M N L L V I S F D R_Y_F S I
BARAVI   E C W T S L D V L C V T A S I E T L C V I A I D R_Y_L A I
B2HUM    E F W T S I D V L C V T A S I E T L C V I A V D R_Y_L A I
B2HAM    E F W T S I D V L C V T A S I E T L C V I A V D R_Y_I A I
B1HUM    E L W T S V D V L C V T A S I E T L C V I A L D R_Y_L A I
HA2_AR   G V Y L A L D V L F C T S S I V H L C A I S L D R_Y_W S V
5HT-2    A I W I Y L D V L F S T A S I M H L C A I S L D R_Y_V A I
5HT-1A   D L F I A L D V L C C T S S I L H L C A I A L D R_Y_W A I
D2-DOP   D I F V T L D V M M C T A S I L N L C A I S I D R_Y_T A V
5HT-1C   P V W I S L D V L F S T A S I M H L C A I S L D R_Y_V A I
SKRBOV   Y F Q N L F P I T A M F V S I Y S M T A I A A D R_Y_M A I
RHBOV    N L E G F F A T L G G E I A L W S L V V L A I E R_Y_V V V

Vj=      7 5 5 6 6 4 3 4 4 6 6 4 4 2 3 6 4 2 4 4 2 2 5 2 1 1 7 4 2
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Fig.7. The sequence alignment for the third putative membrane spanning region. This region is thought to be important in directly binding ligand.

postulated to be important in distorting the structure to allow for ligand binding [7,55,57].

We believe that the theoretical method outlined above allows a good 'first approximation' in the study of the structure of this class of receptors which can then be adapted to take into account the experimental evidence available.

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