Protein Engineering vol.6 no.1 pp.59–64, 1993

Modeling of transmembrane seven helix bundles

Philippe Cronet, Chris Sander and Gert Vriend

EMBL, D-6900 Heidelberg, Germany

Transmembrane seven helix bundles form a large family of membrane inserted receptors and are responsible for a wide range of biological functions. Experimental data suggest that their overall structure is similar to bacteriorhodopsin. We describe here a new approach for the modeling of transmembrane seven helix bundles based on statistically derived environmental preference parameters combined with experimentally determined features of the receptors. The method was used to create a model for the human β_2-adrenoreceptor. This model is physically plausible, is in reasonable agreement with experimental data and may be helpful in planning new receptor engineering experiments.

Key words: β_2-adrenoreceptor/bacteriorhodopsin/protein structure prediction/receptor/sequence–structure alignment

Introduction

G-Protein coupled receptors constitute a large family of membrane spanning receptors of diverse biological function. According to recent estimates, there are several hundred different members of this protein family (Schoffield and Abbott, 1989; Birdsall, 1991; Mollon, 1991). Some of the important members are the α- and β-adrenergic (Lefkowitz and Caron, 1988), muscarinic acetylcholine (Curtis *et al.*, 1989), serotonin (Hartig, 1989), dopamine (Sibley, 1991) and histamine (Birdsall, 1991) receptors, as well as opsin (Kosower, 1988). The latest newcomers are interleukin-8 receptor (Murphy and Tiffany, 1991), endothelian receptor (Lin *et al.*, 1991), the large group of olfactory receptors and, most probably, the taste receptors (Lancet, 1991a,b). All of these receptors are essential for important biological reactions, such as transmission of neural signals through synapses, regulation of phospholipid turnover and controlling the exchange of K^+ and Ca^{2+} through the plasma membrane of cells.

Their activation leads to many basic physiological responses, such as contraction of smooth and striated muscles, gland excretion, induction of complex behavior (affection, depression, aggression), vision, taste and smell. They are thus of major importance in many basic biological functions. Therefore, a better knowledge of their structure–activity relationships is of great scientific and practical interest.

A common structural feature of this family is the occurrence of seven hydrophobic segments, each ~20–30 amino acids in length (Dahl *et al.*, 1991). No structure determination of these receptors has been carried out so far. However, the structure of bacteriorhodopsin (bRh), another protein with the same hydrophobic sequence pattern, has been solved by electron crystallography (Henderson *et al.*, 1990). It is a seven helix transmembrane bundle, with nearest neighbor helices antiparallel to one another. The N-terminus is extracellular, the C-terminus cytoplasmic. Starting from the N-terminus, the helices are arranged counterclockwise in a kidney shaped manner. The precise conformation of the loops has not yet been determined. The native form of bRh is trimeric. Experimental data from deletion mutations, antibody targeting and tryptic digestion experiments suggest that G-protein coupled receptors may have the same topography as bRh. For some receptors, site directed mutagenesis has shown that residues from different transmembrane segments are involved in the ligand binding pocket, just as in bRh (Dixon *et al.*, 1987; Dohlman *et al.*, 1987; Tota and Strader, 1990; Khorana, 1992).

Attempts have been made to derive three-dimensional models of these receptors based on sequence similarity with bRh (Mitchell *et al.*, 1989). However, only very low sequence identity (~9%) is detectable using standard sequence alignment methods. This leads to considerable uncertainty in three-dimensional models based on such alignments.

Here, we describe the modeling of the human β_2-adrenoreceptor (β2adr) by a new approach based on a combination of experimental constraints and theoretical analysis of structural features. The three subtypes β1adr, β2adr and β3adr, together with the α-adrenoreceptors, comprise the important adrenergic receptor family. The distinction of the different subtypes is based on their sensitivity to different agonists and antagonists. β_2-Adrenoreceptors are responsible for a very broad range of tissue specific effects. Activation of β2adr induces relaxation of arterioles, veins and bronchial uterus muscles, decreases stomach and intestine motility, increases insulin secretion and acts on the central nervous system (for review see Mayer, 1980). The active site of the β2adr receptor is situated inside the membrane and no loop residues participate in ligand binding (for review see Tota *et al.*, 1991). Therefore, we focus our attention on the helices of the receptor.

Our modeling procedure involves three levels of detail: overall topography, helix ends and helix phase. First, we assume that β2adr has the same topography as bRh. By topography we mean the relative spatial position of the seven helices and the same order of loop connections between the helices. The assumption is supported by diverse experimental evidence (Dixon *et al.*, 1987; Dohlman *et al.*, 1987; Tota and Strader, 1990) as well as by structural considerations. While in principle there are many different ways of arranging seven helices in a bundle, we note that helix–helix packing of membrane helices in bRh appears to follow simple geometrical rules (e.g. Chothia, 1984), as shown, for example by the very good superimposition (r.m.s. deviation of atomic Cα positions of 2.0 Å over 62 residues) of four bRh helices with the four helices of repressor of primer (Rop) protein (Banner *et al.*, 1987; Eberle *et al.*, 1990). Analogous reasoning was used in modeling of a similar receptor using molecular dynamics (Dahl *et al.*, 1991).

Starting with this topography, the helix ends remain to be defined in the sequence. Hydropathy plots (Kyte and Doolittle, 1982) indicate very approximately the location of the seven hydrophobic regions. However, from the available experimental data on β2adr, the helix ends can be defined with a precision of four residues on average (Table I). The last step in the refinement of the model is to set the correct helix phase or

Table I. Summary of the experimental information about the topography of the β2adr

Residues	Location	Reference
1–30	N-terminal segment	Dixon *et al.* (1987)
58–71	could be intra-loop 1	O'Dowd *et al.* (1988)
79	in helix 2	Strader *et al.* (1988)
	affects agonists binding	Strader *et al.* (1987)
99–102	in extra-loop 1	Dixon *et al.* (1987)
106	end of extra-loop 1	Dixon *et al.* (1987)
	makes an S–S bond with C184	Strader *et al.* (1988)
113	helix 3	
	11 Å in the membrane and directly involved in ligand binding	Tota and Strader (1990)
131–149	cyto-loop 2	O'Dowd *et al.*, (1988)
175–186	extra-loop 2	Dohlman *et al.* (1987)
184	S–S bond with C 106	Dixon *et al.* (1987)
204, 207	helix 5	Strader *et al.* (1989)
215–220	helix 5	Dixon *et al.* (1987)
221–272	intra-loop 3	Dixon *et al.* (1987)
290	helix 6	Tota and Strader (1990)
	involved in agonists binding	Tota *et al.* (1991)
301–303	extra-loop 3	Dixon *et al.* (1987)
318–323	helix 7	Strader *et al.* (1987)
	Asn318 involved in agonist binding	
330	beginning of C-terminal	O'Dowd *et al.* (1988)
340–418	C-terminal chain	Dixon *et al.* (1987)

rotational orientation of the helices. The phase of a helix determines whether a particular residue is in contact with lipid, with another helix or points toward the central channel. How can the helix phase be predicted? Assuming that the topography and packing of the helices are the same as in bRh, the distribution of the residues in β2adr with respect to the phospholipid bilayer and to the central channel of the receptor should be consistent with the residue distribution found in bRh. The key idea of our method is to quantify the preferred environment for the 20 different amino acid types in seven transmembrane helix bundles in terms of preference parameters. The parameters are derived from the structure of bRh and the sequences of its close homologs. The parameters are then used to determine the optimal fit of the β2adr sequence onto the bRh structural template. The main problem with modeling studies that are based on bRh is the limited resolution of the data in the direction perpendicular to the membrane (Henderson *et al.*, 1990). Indeed, refinement of the structure made clear that, for example, helix 4 has to be shifted by +3 Å in the *z*-direction (R.Henderson, personal communication). One of the main advantages of our method is that it is not very sensitive to such problems.

Materials and methods

Determining environmental preferences

Interactions of side-chains with their environment are a basic determinant of tertiary structure. In order to derive preference parameters for different contact environments, we need a discrete structural environment. The local environment of each residue in bRh is determined by its position in the helix. This position can be defined by the angle α between the direction in which the residue points, as given by the vector from the helix axis to the Cβ atom, and the direction in which the nearest adjacent helix is located (Figure 1A and B), as given by the vector from the axis of one helix to that of its nearest neighbor. We define $\alpha = 0$ when a residue points to the axis of the adjacent helix. By convention, the angle is positive if the side-chain points into lipid and negative if it points into the internal cavity.

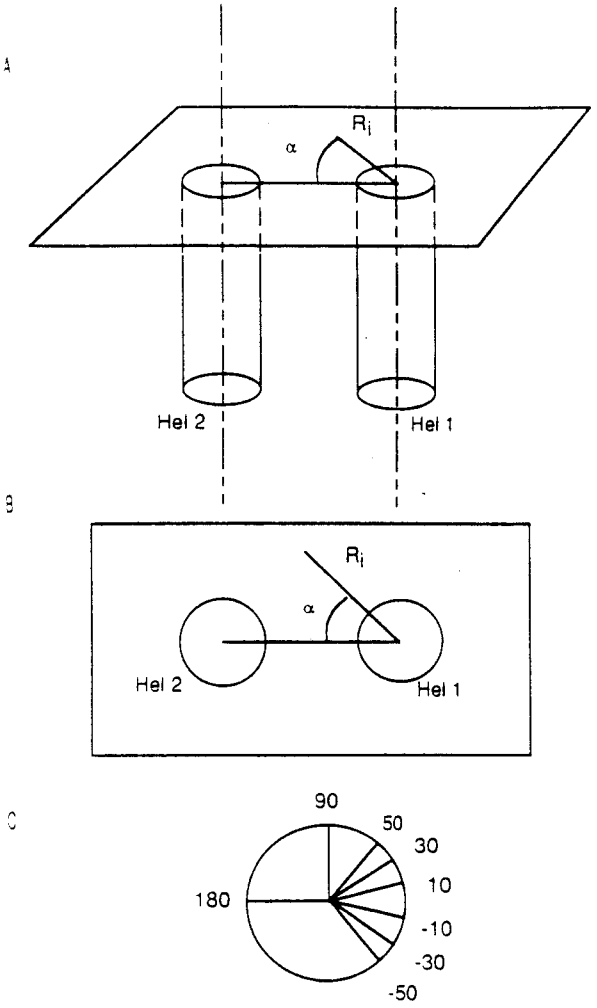


Fig. 1. (**A** and **B**) Definition of residue angular orientation in the bRh helix bundle. R_i is the residue position. (**C**) Schematic illustration of the angle ranges chosen to describe residue orientation in bRh helices. Negative angles correspond to residues pointing into the central channel.

Measurements of residue orientation were done on the trimer of bRh with the lipid mapped around the trimer using a surface mapping option of WHAT IF (Vriend, 1990).

As there are only 170 residues in bRh helices and eight angular orientations to be sampled, the residue preferences are subject to statistical noise. In order to reduce statistical noise, we have exploited information from proteins homologous to bRh. The Swiss-Prot (Bairoch and Boeckmann, 1991) database was searched using the program MaxHom, with higher weights on conserved positions, and retaining only those sequences with similarity to bRh higher than the threshold for structural homology (Sander and Schneider, 1991). Only parts of the homologous sequences without insertions and deletions in helices were used. The final data set had 526 residues from four sequences.

Eight angular ranges were used to characterize the different environmental features found along the helices in the membrane bundle (i.e. the transition from a completely hydrophobic environment to the hydrophilic internal cavity) (Figure 1C). The preference or information parameter $P_{\alpha,r}$ of a residue of type r for the angular range around α is defined as follows

$$P_{\alpha,r} = \ln(N_{\alpha,r} \cdot N_t / N_\alpha \cdot N_r)$$

where $N_{\alpha,r}$ is the number of residues of type r in a particular

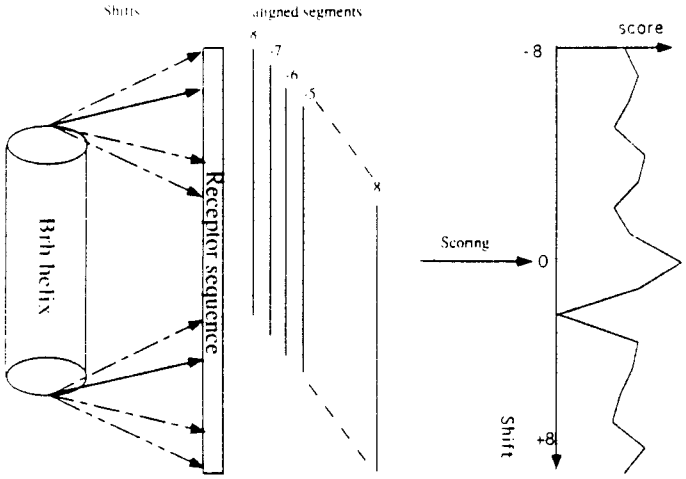


Fig. 2. Illustration of the alignment and scoring procedure. A putative transmembrane segment of the receptor is threaded (shifted) through a bRh helix and is scored according to the sum of residue environmental preferences. No insertions or deletions are allowed. The score is plotted as a function of sequence shift.

angular range around α, N_r is the total number of residues of type r, N_α is the total number of residues in a particular α range, N_t is the total number of residues in the data set and ln is the natural logarithm. For a given residue orientation and residue type, $P_{\alpha,r}$ quantifies how well a particular residue fits a particular structural position. The set of preferences $P_{\alpha,r}$ can be used to evaluate how well a new sequence fits into the bRh structural template.

Scoring sequence–structure alignments

In order to evaluate the compatibility between a new sequence and the bRh structural template, the membrane receptor sequence is threaded into the bRh structure and each residue in a helix is scored according to its environmental preference (Figure 2). The ends of the transmembrane regions are roughly defined according to a combination of hydropathy analysis and experimental data (see, for example, Table I). The correct phase of the helices is then only searched within two helical turns, upstream and downstream of these ends. This is done by shifting the aligned sequence ± 8 positions relative to the original alignment (shift = 0). The score of each residue is $P_{\alpha,r}$. The sum of the single residue scores $P_{\alpha,r}$ over all helix positions gives a measure of the quality of the alignment. Note that this sum is equivalent to the logarithm of a product of relative probabilities. Each helix is considered separately and no insertions/deletions are introduced. Alignments which place experimentally known loop residues onto a helical segment were of course excluded. The alignment with the highest score that is consistent with the experimental features listed in Table I is selected.

Modeling the receptor

The residues corresponding to the final alignment in each helix are then placed into the bRh structural template and atomic overlaps are removed by an iterative search of side-chain dihedral χ angles in discrete rotamer space (WHAT IF; Vriend, 1990). A final refinement of the model is made with a 2400 step steepest descent energy minimization using the consistent valence force field (CVFF; Dauber-Osguthorpe *et al.*, 1988), in vacuum and with fixed backbone.

Graphical inspection and manipulations were carried out using the programs Insight (Dayringer *et al.*, 1986) and WHAT IF (Vriend, 1990). Energy minimization was carried out using the program Discover (Biosym). Data were analyzed using a program specifically written for this purpose.

The program and the model are available on request.

Results and discussion

Building the preference matrix

Three proteins have unambiguous sequence similarity with bRh in the helical regions: archerhodopsin and two halorhodopsins (Swiss-Prot codes: baca__halsa, bach__natph and bach__halsp) with respectively 60, 36 and 34% residues identical to bRh. For globular proteins, this level of identity implies essentially identical structures (Chothia and Lesk, 1986; Sander and Schneider, 1991). The residues in these homologous proteins can therefore be considered as having the same angular distribution as those in bRh. In a larger data set extended by homology, there are on average 3.1 residue counts for each angular orientation in the bRh structure template. The resulting environmental preference parameters are in Table II. Most of the residue types have a clear preference for one or several bins (no histidine residues were found in the data set). For example, tyrosine and tryptophan residues are more frequent in the bin with $-180 < \alpha < -50$ and serine has a clear preference for the interfaces between the helices ($\alpha \approx 0$).

Testing the method

Amphipathy has been suggested to be a determinant of insertion and packing in phospholipid membranes (Eisenberg *et al.*, 1984). In order to check if our environmental parameters reflect more information than a mere outside/inside (hydrophobic lipid/polar channel) residue distribution, the hydrophobic moment vectors of bRh were calculated. No obvious correlation between the orientation of the hydrophobic moment vectors and the orientation of the helices with respect to the lipid is obtained (Figure 3). For example, the hydrophobic moment vector of the fourth helix (bottom right in Figure 3) points into the central cavity rather than into the more hydrophobic lipid. A look at the three-dimensional structure reveals many hydrophobic residues unexpectedly facing the inside of the protein as well as some hydrophilic residues pointing toward the lipid. In spite of the fact that the transmembrane regions can be delineated in the sequence using hydrophobic moment values, this test shows that more detailed information is needed to properly orient a helix with respect to its environment in the seven helix bundles.

An additional problem of the usage of hydrophobic moment vectors, or conservation moment vectors (Komiya *et al.*, 1988), is that the vector will by definition not determine the exact phase of the helix, but rather indicate a general tendency. Also, for example, for conservation moments, it is not clear where they should point. Should the vectors point into the central cavity because that is where the conserved active site residues are, or should they point to the nearest neighbor helix because the helix–helix interface has the most structural constraints?

The environmental preference parameters derived from bRh and three homologs are based on a very small number of residues and as such there is the danger that they are not representative of other unrelated proteins. Evidence for the validity of the method comes from application to bovine rhodopsin. Despite both being rhodopsins, bRh and bovine rhodopsin have no sequence similarity (5–12% sequence identity). Bovine rhodopsin has been studied extensively and some important membrane embedded residues have been mapped experimentally (Nakayama and Khorana, 1991) as well as the positions of the residues that interact with the retinal. The bovine rhodopsin sequence was fitted into the bRh structure using our method. Reassuringly, the

Table II. Scoring table of residue environmental preferences $P_{\alpha,r}$

Limits		ALA	CYS	ASP	GLU	PHE	GLY	HIS	ILE	LYS	LEU	MET	ASN	PRO	GLN	ARG	SER	THR	VAL	TRP	TYR
−10	10	0.4	0.0	0.1	0.0	−1.3	1.2	0.0	0.0	−0.1	−0.3	0.5	0.0	0.0	0.0	0.0	0.9	0.4	−0.6	0.0	0.4
10	30	−0.1	1.4	0.0	0.0	−0.1	−0.1	0.0	0.6	−0.6	0.0	0.0	0.0	0.8	0.0	0.3	−0.1	0.0	0.1	−0.2	−0.7
30	50	−0.4	0.8	0.1	0.5	0.0	0.4	0.0	−0.3	0.8	0.5	−0.3	0.1	0.6	0.0	−0.3	−1.1	−0.8	0.4	−0.2	−1.4
50	90	−0.6	0.0	−0.4	−0.8	0.6	−0.1	0.0	0.5	−0.7	0.0	−1.2	1.0	0.0	0.0	0.6	0.1	−0.5	0.1	0.6	−0.5
90	180	0.8	0.0	0.0	0.0	−0.4	0.0	0.0	0.6	0.0	−0.2	0.0	0.7	0.0	0.0	0.3	−0.5	−0.2	0.7	0.0	0.4
−30	−10	−0.1	0.0	1.1	0.0	0.1	0.0	0.0	−1.2	0.0	−0.9	1.3	0.0	0.9	0.0	0.0	1.0	0.6	−0.7	0.8	0.0
−50	−30	0.5	0.0	0.0	0.8	0.8	−0.1	0.0	−0.3	0.0	−0.6	0.8	0.0	0.2	0.0	0.0	−0.4	0.4	−0.3	0.0	0.2
−180	−50	0.0	0.0	1.4	0.6	0.0	0.0	0.0	0.0	1.4	0.5	−0.5	0.0	0.0	2.4	0.9	0.0	−0.3	−1.6	1.1	1.0

This table is used to evaluate the compatibility of sequences with the bRh structure in a given alignment. Limits: angular ranges of the angle α (Figure 1).

sequence−structure alignment proposed by Nakayama and Khorana, which is based on chemical knowledge rather than on computational techniques, corresponds to a local maximum in the plot of score versus shift for all helices. In helices B, D, E and F their alignment corresponds to the highest score, in helices C and G to the second highest score and in helix A to the third highest score.

Modeling the β_2-adrenoreceptor

These tests strongly suggest that our method is able to detect the phase of helices in a bRh-like structure. We were thus encouraged to apply our modeling procedure to β2adr. Extensive experimental studies have been carried out on the topography and on functionally important residues of this receptor (Table I). The correct phase of each helix was searched for within the experimentally defined limits using the preference parameters as the main guide, as described in Materials and methods.

In order to improve the precision of the alignment of the β2adr sequence in the bRh structure, the Swiss-Prot database (Bairoch and Boeckman, 1991) was scanned for sequences similar to β2adr using the program MaxHom (Sander and Schneider, 1991). No insertions or deletions were allowed in helices. Thirty-three sequences were deemed to be homologous to β2adr in the transmembrane regions. The sequence−structure alignment to bRh was applied to all 33 sequences. Figure 4 shows the results obtained for the seven putative helices, as plots of score versus shift, averaged over all 33 sequences homologous to β2adr.

The model (Figure 5a) was built from the alignments corresponding to the highest score that is consistent with the experimental features listed in Table I. It should also be noted that evolutionary pressure on residues in transmembrane segments is markedly different from what is found in globular proteins. Therefore, the usual rules of thumb, for example, that Cys, Trp or Pro have to be conserved, do not hold in these transmembrane helices (L.Oliveira, personal communication). The overall alignment is given in Figure 5(b). A detailed discussion for each of the helices follows below.

Helix A. The highest score is obtained with the alignment of T25−F49 to the first helix of bRh. However, residues T25−D29 are part of extracellular loop 1 (Dixon *et al.*, 1987). We therefore chose the second highest score that does not incorporate any loop residues in the helix and we propose helix 1 to consist of residues V31−I55.

Helix B. The sequence alignment showing the highest score would place W99−F101, which are part of extracellular loop 2, in the helix. As for the first helix, the alignment corresponding to the highest score which does not include known loop residues was chosen. The alignment includes L75−W99 in the helix and corresponds to the third highest score. Residue D79, which is involved in agonist binding, points into the central channel close to the cytoplasmic interface. It is thus not situated at the same height as other residues of the putative active site (see helices 3 and 5). However, it has been suggested that it might not be directly bound to agonists but most likely would be involved in stabilization of an agonist-induced conformation (Strader *et al.*, 1987).

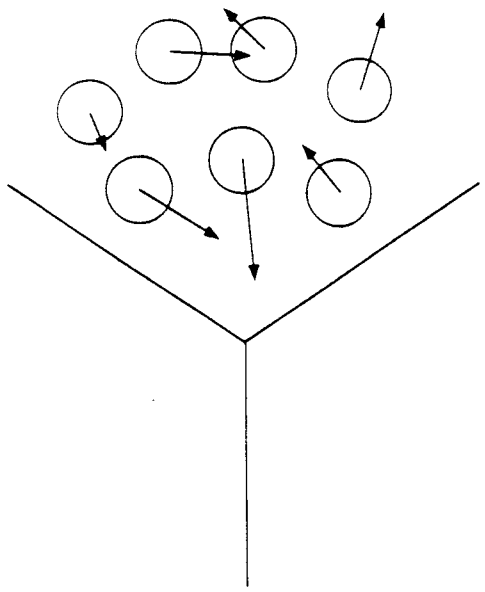


Fig. 3. Section through the bRh monomer parallel to the membrane surface at half height, with the orientation of the hydrophobic moment vector (Eisenberg *et al.*, 1984) indicated by arrows. The moments do not consistently point into the lipid side of the bRh helical bundle, so they cannot be used as a simple criterion of correct phasing. The V-shaped line symbolizes the interface with the other monomers in the bRh trimer.

Helix C. According to the highest score, F108−A134 would form the third helix of β2adr. In that alignment, residue D113, which is known to bind the amino group of the ligand, points into the cavity and lies close to the extracellular interface. However, fluorescence measurements suggest that the ligand lies 11 Å from the surface inside the membrane (Table I). The position of D130 reinforces our feeling that this alignment does not fit the experimental data. This residue participates in the receptor coupling to the G protein (Mitchell *et al.*, 1989), but lies 7 Å inside the membrane according to this alignment.

The sequence alignment corresponding best to the experimental constraints (Table I) has the second highest score. Helix 3 thus consists of residues F104−D130. In this alignment, D113 is brought to the same height as the other active site residues and D130 lies near the membrane interface.

Helix D. Helix 4 was built according to the highest score. It involves residues T146−L167. Although the N-terminal turn is quite hydrophilic, no other constraints would justify another choice.

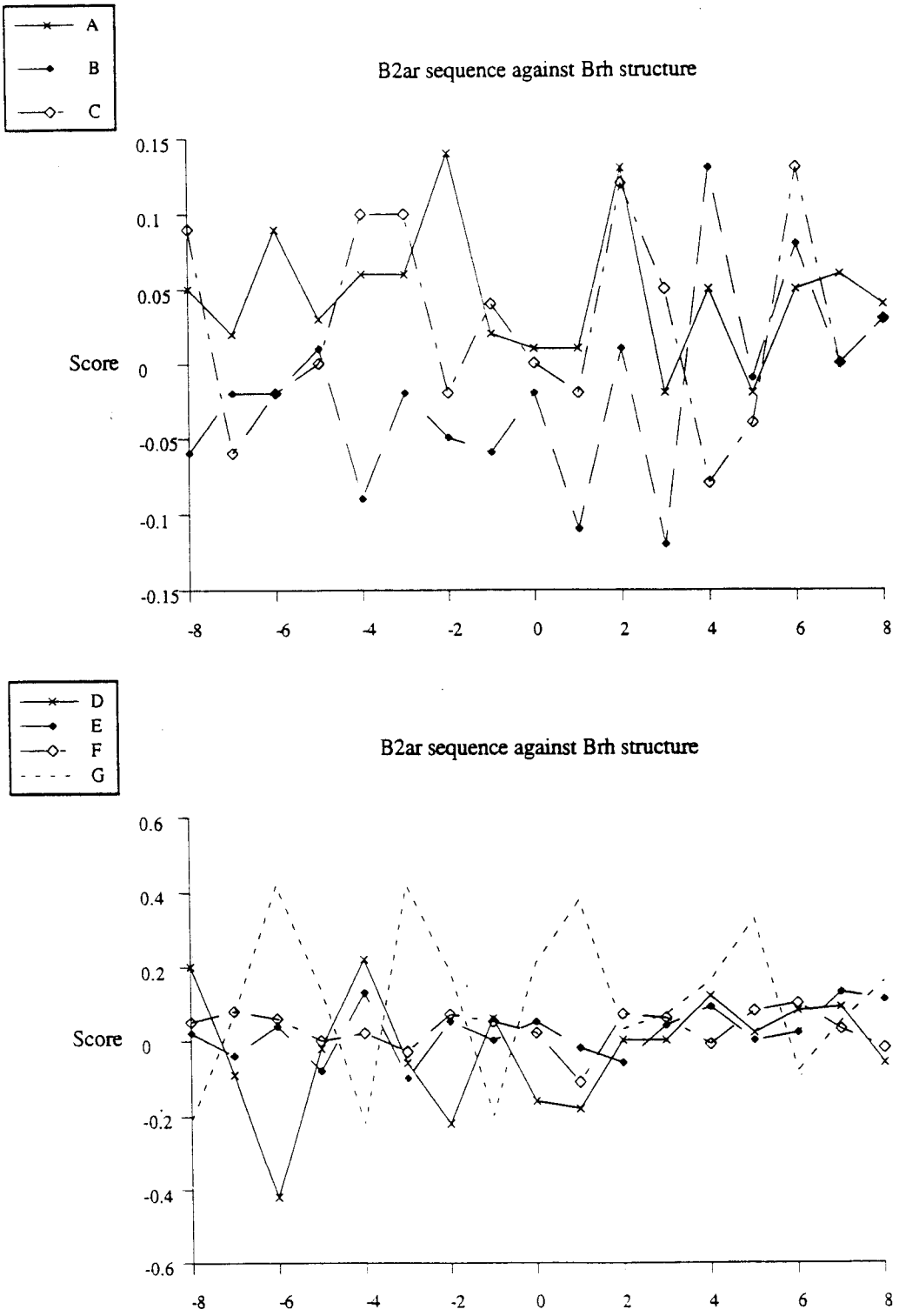


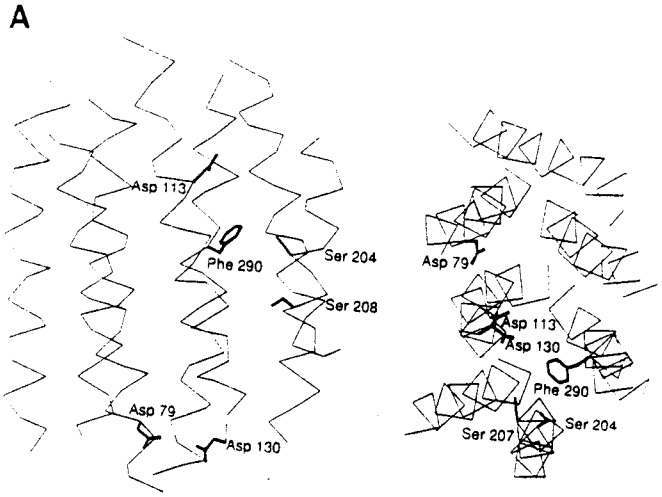
Fig. 4. Plots of the scores obtained when threading the sequence of β2adr through the bRh structure as a function of threading shift. Symbols A–G correspond to the seven transmembrane helices.

Helix E. This helix was also built according to the highest score. Residues S204 and S207, which are part of the active site, point into the central cavity, approximately two turns of a helix inside the membrane. This corresponds to the expected active site position (Strader *et al.*, 1989).

Helix G. The alignment corresponding to T274–Q299 was chosen as it has the highest score without including known loop residues in the helix. This is the third absolute score for this helix. It also positions F290 at the active site level pointing into the central cavity, which is consistent with experimental results (Tota *et al.*, 1991).

Helix F. As for the previous helix, the alignment was chosen so as not to include any loop residues; it has the highest score and involves residues E306–S329 .

The method determined the phase of all helices, i.e. for all residues it is predicted whether they point towards the lipid,



B

```
bRh     P E W I W L A L G T A L M G L G T L Y F L V K G M
b2adr   V W V V G M G I V M S L I V L A I V F G N V L V I

bRh     D A K K F Y A I T T L V P A I A F T M Y L S M L L
b2adr   L A C A D L V M G L A V V P F G A A H I L M K M W

bRh     E Q N P I Y W A R Y A D W L F T T P L L L L D L A L L
b2adr   F W C E F W T S I D V L C V T A S I E T L C V I A V D

bRh     G T I L A I V G A D G I M I G T G L V G A L
b2adr   T K N K A R V I I L M V W I V S G L T S F L

bRh     W W A I S T A A M L Y I L Y V L F F G F T
b2adr   N Q A Y A I A S S I V S F Y V P L V I M V

bRh     E V A S T F K V L R N V T V V L W S A Y P V V W L I
b2adr   T L G I I M G T F T L C W L P F F I V N I V H V I Q

bRh     N I E T L L F M V L D V S A K V G F G L I L L R
b2adr   E V Y I L L N W I G Y V N S G F N P L I Y C R S
```

Fig. 5. (**A**) Side (left) and top (right) view of the β2adr model Cα trace. Labeled residues participate in binding agonists or antagonists (see Table I). Drawn using WHAT IF (Vriend, 1990). (**B**) Sequence alignment between bRh and β2adr on which the three-dimensional model is based. Each helix is shown separately. b2adr = β2adr.

towards another helix or into the central cavity. However, as helices have a periodicity of 3.6 residues, additional information is still needed to determine where the residue is located: at the calculated position, or three or four residues further up or down in the helix.

The resulting final model avoids charged residues pointing into the lipid and fits all known experimental features of the receptor (see Table I). We thus feel confident that this model can be used as a basis for drug design. Based on the model specific point mutation experiments can be proposed in order to improve our knowledge of the structure–activity relationship of the receptor. For example, both N185 and N318 lie at the level of the ligand binding pocket and could be involved in ligand binding.

Conclusions

We propose here a method for modeling of seven transmembrane helix bundles. This hybrid method combines statistically derived environmental preference parameters with experimentally determined features of the receptors. With the present limited amount of chemical and structural data available, neither chemical constraints nor preference parameters alone are sufficient. Taken together, however, they yield a model for the β_2-adrenoreceptor that makes physical sense and is consistent with experimental data. We expect that this 'knowledge based' prediction method will be of practical use for modeling the structure of this membrane receptor family.

Acknowledgements

We thank David Thomas for his help in the evaluation of the method and for discussion of the results. We also thank T.Ceska, G.Dijkstra, W.Kuehlbrandt, M.G.Caron, A.P.Czernilofsky, L.Oliveira, and A.Paiva for critical comments. We thank R.Henderson for providing us with information about the refined bacteriorhodopsin coordinates prior to publication. This work was supported in part by a grant from the EC Bridge program.

References

Bairoch,A. and Boeckmann,B. (1991) *Nucleic Acids Res.*, **19**, 2247–2249.
Banner,D.W., Kokkinis,M. and Tsernoglou,D. (1987) *J. Mol. Biol.*, **196**, 657–675.
Birdsall,N.J.M. (1991) *TIPS*, **12**, 9–10.
Chothia,C. (1984) *Annu. Rev. Biochem.*, **53**, 537–572.
Chothia,C. and Lesk,A.M. (1986) *EMBO J.*, **5**, 823–826.
Curtis,C.A.M., Wheatley,M., Bansal,S., Birdsall,N.J.M., Eveleigh,P., Pedder,E.K., Poyner,D. and Hulmes,E.C. (1989) *J. Biol. Chem.*, **264**, 489–495.
Dahl,S.G., Edvarsen,O. and Sylte,I. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 8111–8115.
Dauber-Osguthorpe,P., Roberts,V.A., Osguthorpe,D.J., Wolff,J., Genest,M. and Hagler,A.T. (1988) *Protein Struct. Funct. Genet.*, **4**, 31–47.
Dayringer,H.E., Tramontano,A. and Fletterick,R.J. (1986) *J. Mol. Graph.*, **4**, 82–87.
Dixon,R.A.F., Sigal,I.S., Candelor,M.R., Register,R.B., Scatterwood,W., Rands,E. and Strader,C.D. (1987) *EMBO J.*, **6**, 3269–3275.
Dohlman,H.G., Bouvier,M., Benmovic,J.L., Caron,M.G. and Lefkowitz,R.J. (1987) *Biol. Chem.*, **262**, 14282–14288.
Eberle,W., Klaus,W., Ceasareni,G., Sander,C. and Rösch,P. (1990) *Biochemistry*, **29**, 7402–7407.
Eisenberg,D., Schwartz,E., Komaromy,M. and Wall,R. (1984) *J. Mol. Biol.*, **179**, 125–142.
Hartig,P.R. (1989) *TIPS*, **10**, 64–69.
Henderson,R., Baldwin,J.M., Ceska,T.A., Zemlin,F., Beckman,E. and Downing,K.H. (1990) *J. Mol. Biol.*, **213**, 899–929.
Khorana,H.G. (1992) *J. Biol. Chem.*, **267**, 1–4.
Komiya,H., Yeates,T.O., Rees,D.C., Allen,J.P. and Feher,G. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 9012–9016.
Kosower,E.M. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 1076–1080.
Kyte,J. and Doolittle,R.F. (1982) *J. Mol. Biol.*, **157**, 105–132.
Lancet,D. (1991a) *Nature*, **351**, 275–276.
Lancet,D. (1991b) *Nature*, **353**, 799–800.
Lefkowitz,R.J. and Caron,M.G. (1988) *J. Biol. Chem.*, **263**, 4993–4996.
Lin,H.Y., Harris,T.L., Flannery,M.S., Aruffo,A., Kaji,E.H., Gorn,A., Kolakowski,L.F., Lodish,H., Jr and Goldring,S.R. (1991) *Science*, **254**, 1022–1024.
Mayer,S.E. (1980) In Goodman,L.S. and Gilman,A. (eds), *The Pharmacological Basis of Therapeutics*. Macmillan Publishers, New York, pp. 56–90.
Mitchell,T.J., Tute,M.S. and Webb,G.A. (1989) *J. Comput. Aid. Mol. Des.*, **3**, 211–223.
Mollon,J. (1991) *Nature*, **351**, 696–697.
Murphy,P.M. and Tiffany,H.L. (1991) *Science*, **253**, 1280–1282.
Nakayama,T.A. and Khorana,H.G. (1991) *J. Biol. Chem.*, **266**, 4269–4275.
O'Dowd,B.F., Hantowich,M., Regan,J.W., Leader,W.M., Caron,M.G. and Lefkowitz,R.J. (1988) *J. Biol. Chem.*, **263**, 15985–15992.
Sander,C. and Schneider,R. (1991) *Protein Struct. Funct. Genet.*, **9**, 56–68.
Schoffield,P.R. and Abbott,A. (1989) *TIPS*, **10**, 208–212.
Sibley,D. (1991) *TIPS*, **12**, 7–9.
Strader,C.D., Sigal,I.S., Register,R.B., Candelor,M.R., Rands,E. and Dixon,R.A.F. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 4384–4388.
Strader,C.D., Sigal,I.S., Candelor,M.R., Rands,E., Hill,W.S. and Dixon,R.A.F. (1988) *J. Biol. Chem.*, **263**, 10267–10269.
Strader,C.D., Candelor,M.R., Hill,W.S., Sigal,I.S. and Dixon,R.A.F. (1989) *J. Biol. Chem.*, **264**, 13572–13578.
Tota,M.R. and Strader,C.D. (1990) *J. Biol. Chem.*, **265**, 16891–16897.
Tota,M.R., Chandelor,M.R., Dixon,R.A.F. and Strader,C.D. (1991) *TIPS*, **12**, 4–6.
Vriend,G. (1990) *J. Mol. Graph.*, **8**, 52–55.

Received on June 25, 1992; accepted on October 28, 1992