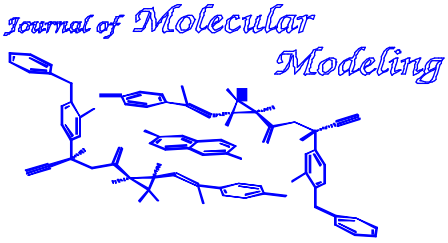
J. Mol. Model. **1997**, *3*, 70 – 77



Identification of Helical Packing Motifs Common to Bacteriorhodopsin and G Protein-Coupled Receptors

M. Germana Paterlini, Thomas G. Metzger, Philip S. Portoghese, and David M. Ferguson*

Department of Medicinal Chemistry and Minnesota Supercomputer Institute, University of Minnesota, 308 Harvard Street SE, Minneapolis, MN, 55455, USA; Fax: (612) 626 4429 (ferguson@quinn.medc.umn.edu)

Received: 26 August 1996 / Accepted: 17 December 1996 / Published: 7 February 1997

Abstract

A sequence analysis and comparison of transmembrane helices in bacteriorhodopsin (BR) and G protein-coupled receptors (GPCRs) is presented to identify potential regions of homology across protein families. The results show a common pattern of residues is conserved within the interhelical contact regions of BR that fit a knob-into-hole structural motif previously postulated for globular proteins and photosynthetic reaction centers. Based on an alignment of conserved prolines in transmembrane helices, it is inferred that analogous helix packing arrangements are possible in the rhodopsin-like GPCRs. Molecular models of GPCR helices V and VI indicate these interactions occur between aromatic and hydrophobic residues flanking the highly conserved prolines in these sequences. A similar packing arrangement is shown to occur in the X-ray structure of the melittin which also displays a unique pairing of proline-linked helices. The contact pattern identified is further applied to predict the packing of pairs of proline-containing helices in the pheromone-like and cAMP GPCRs. A potential role in stabilizing structure formation is also suggested for the contacts. The results and conclusions are supported by recent biophysical studies of zinc binding to kappa-opioid receptor mutants.

Keywords: Helix-helix interaction, proline-containing helices, bacteriorhodopsin, G protein-coupled receptor.

Introduction

An understanding of G protein-coupled receptor (GPCR) function at the molecular level depends heavily on the determination of a detailed 3-dimensional structure for this protein family. Information on the structure of GPCRs, however, has been difficult to obtain. The low resolution density map of rhodopsin, and the subsequent assignment of the transmembrane (TM) helices, has suggested a TM packing arrangement similar to, but not identical to that of bacteriorhodopsin (BR) [1,2]. Although BR is often used as a structural template for GPCR model building, the distant sequence relationship of these proteins is problematic. The helical distortions caused by the proline residues in TM helices II, III, and VI of BR can not be reconciled with GPCR sequences of the rhodopsin family, where highly conserved prolines are found in TM helices IV through VII [3]. In the past, this shortcoming has either been ignored or remedied using averaged templates from the proline-containing helices of BR [4]. An alternative approach has also been suggested that uses non-sequential alignments of individual TM sequences. Pardo et al. proposed a GPCR alignment based on exon shuffling that radically reorganized the helix ordering in the TM domain [5]. Although the approach paired prolines in BR helix III and GPCR helix V, the overall pack-

* *To whom correspondence should be addressed*

ing scheme suggested has since been discounted. More recently, Metzger et al. reported an analysis of conserved residues in retinal and the rhodopsin-like GPCRs that treated each TM helix as an individual domain [6]. The resulting alignment allowed direct structural templates for GPCR helices V and VI to be taken from the crystallographic structure of BR helices III and II, conserving the position and conformational effects of the prolines in these structures. Based on sequence analyses, this pairing was also shown to preserve the sequential ordering of the helices found in BR. The predicted arrangement has been supported by recent site directed mutagenesis studies of the muscarinic receptor as well as biophysical studies of zinc binding to mutant GPCRs [7,8].

While non-sequential alignments may address the conformational problems associated with proline-containing helices, the sequence identity is minimal. Helix-helix interactions in BR and the GPCRs, however, may contain other commonalities shared with broader classes of globular and transmembrane proteins. In helical proteins, sequence and structural analyses have indicated a common packing motif may exist that pairs hydrophobic residues in the *a* and *d* positions of complimentary helices, forming what has been referred to as "knob-into-hole" contacts between helices (where *a* and *d* refer to positions in the *a-g* heptad repeat of alpha-helices) [9-11]. A schematic representation of this contact pattern for both parallel and antiparallel helices is shown in figure 1. Within this general scheme, the repeating hydrophobic residues at positions *a-d-a* pair with complimentary residues *a-d-a* for parallel or *d-a-d* for antiparallel orientations. Contacts are also possible with adjacent residues at position *e* and *g* of the helices. The overall arrangement of these residues on the face of each helix produces the so-called knobs and holes of the interaction.

A similar scheme has also been proposed based on a ridge into groove model for helix packing [12]. Although residue contacts follow the same *a-d-a* pattern (i-3, i, i+4 in the nomenclature of ref.12), the *a-d-a* hydrophobic repeat is not a requirement of the model. The characterization of ridge into groove packing is therefore more dependent on structural data which can limit its application in sequence analysis and structure prediction. (See ref. 13 for additional comments in this regard.)

Although knob-into-hole contacts have been observed in transmembrane proteins in previous studies (e.g. photosynthetic reaction centers or RCs [14]), a similar analysis has not yet been extended to GPCR sequences. These receptors, as well as BR, also contain a rare pairing of proline-containing helices. While various structural and functional roles have been suggested for the existence of these conserved prolines, the importance of this unique pairing has not yet been established. In the following sections, we review the BR structure in terms of interhelical contacts and helix packing effects and describe possible homologies to GPCRs. The results are applied to model the supersecondary structure of helices V and VI for a representative GPCR sequence. An analyses of this structure is also performed to gain insight to the overall packing of helices in the TM domain and to the potential role knob-into-hole interactions may play in structure formation.

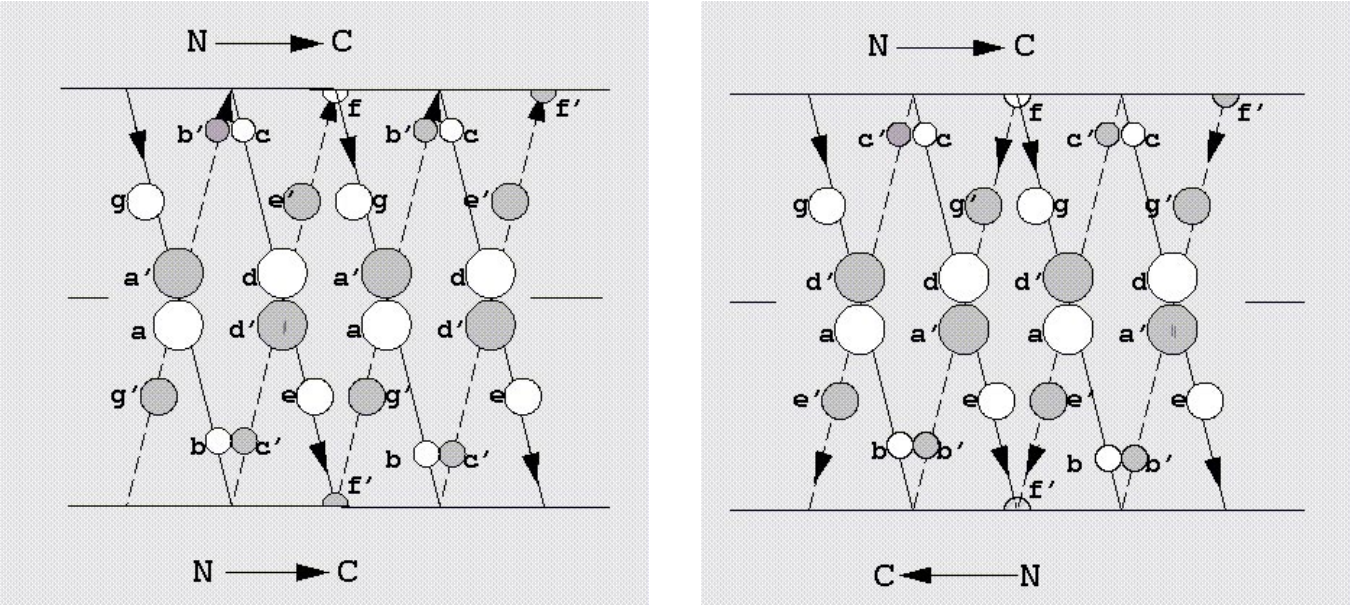


Figure 1. *2-dimensional net illustrating the a-d notation for two alpha-helices in a coiled coil arrangement ;* **(a)** *parallel helices (after ref. 11);* **(b)** *antiparallel helices.*

Methods

The atomic coordinates for BR [15] and melittin [16] were taken from the Brookhaven Protein Databank [17] and displayed graphically using the Midas Plus software package [18]. Interhelical van der Waals contacts were determined by visual inspection of the crystallographic structure. The helix crossing angle, Omega, and distance of closest approach, D, was also determined for these structures using the method of Chou et al. [19] (equations 20-21 for Omega and 25-26 for D of reference 19). The BR structure was further used to model build helix packing for TM helices V and VI of the kappa-opioid receptor using a non-sequential alignment to BR helices III and II, respectively. This alignment procedure is described elsewhere [6]. The resulting structure was refined with molecular mechanics calculations using the Cornell et al. force field [20]. The helices were capped with acetyl and N-methyl groups (as appropriate) and subsequently minimized with the SANDER program of AMBER [21]. A non-bonded cutoff of 12 angstroms was applied with a constant dielectric of 1.0. Least squares fitting of the amino acid backbone atoms of the minimized structure to the BR template was performed using the ANAL module of AMBER.

The conservation of amino acids involved in interhelical contacts across the rhodopsin- like GPCR family was determined by a manual inspection of sequence alignments. In all, 332 sequences were taken from the OWL database [3] and aligned according to the conserved proline residues of helices V and VI. Nonidentical sequences were further analyzed using the PERCAN suite of programs [22]. Fourier transform analyses of lipid substitution patterns for 226 helix V and 204 helix VI sequences were performed using the SUB routine. Direction vectors were taken from the window of maximum periodicity as suggested by Donnelly et al. [22].

Figure 2. *Schematic of interhelical contacts for helices I to VII in BR, as determined by visual inspection of van der Waals interactions. Interacting residues are in bold face and shadowed. Arrows indicate chain direction.*

Results and Discussion

A pairwise analysis of helix contacts in BR is given in figure 2. This figure suggests a network of residue contacts may exist between amino acids located three, four, or seven residues apart linking helices. Although contacts of this type have been postulated in the past for BR [10], the structural details of these interactions were not reported since the crystallographic structure was not known at that time.

The residues identified here suggest these contacts are dominated by hydrophobic and aromatic interactions, similar to those seen in photosynthetic reaction centers and other helical proteins [14,23]. These pairs stack along an imaginary plane parallel to both helix axes, filling, what could be referred to as the helical grooves of the receptor. The helix crossing angle and distance of closest approach for helix pairs in BR, shown in Table 1, are also consistent with previous analyses. In past studies, these values have been used to characterize the structural interaction as either a 3-4 ridge- into-groove or the similar knob-into-hole type of contact [9,12]. The values reported in Table 1 overlap the ideal values for both types of interactions.

An exception is noted, however, for helices III and IV which show a positive tilt angle of 164 degrees (or -20). This may be a consequence of the large pro-induced kink in helix III that may alter the packing requirements of this helix. The reverse tilting may also be due to the unique position of helix IV in the overall structure of BR. Helices II through IV have been shown to lie perpendicular to the plane of the membrane while helices V through VII are slightly tilted. Helix IV is therefore at the interface of the two domains which may alter local packing effects. Regardless of the origin, the interaction suggests both positive and negative tilt angles may be possible for this helix contact pattern. Figure 3 further outlines the residue contacts between helices II and III of BR. Both helices contain a highly conserved proline residue at approximately the same depth within the TM domain. While such an occurrence would be considered rare in globular proteins, the presence of proline residues in transmem-

Table 1. *Interhelical angles and distance of closest approach between helices in Bacteriorhodopsin and melittin.*

Bacteriorhodopsin			
TM	TM	Ω[a] degrees	D[a] Å
I	II	-156	8.0
I	III	32	16.0
I	IV	-161	22.5
I	V	30	29.9
I	VI	-162	20.0
I	VII	11	9.6
II	III	-179	9.5
II	IV	-6	19.1
II	V	-157	20.6
II	VI	18	16.2
II	VII	-160	10.1
III	IV	164	9.6
III	V	20	10.6
III	VI	-159	10.8
III	VII	25	12.6
IV	V	-154	8.3
IV	VI	19	12.9
IV	VII	-162	16.7
V	VI	-168	9.7
V	VII	19	19.8
VI	VII	-172	10.0

Melittin			
TM	TM	Ω[a] degrees	D[a] Å
I	II	-162	10.5
I	III	59	10.2
I	IV	-123	7.1
II	III	-123	7.1
II	IV	61	10.8
III	IV	-162	10.5

[a] Calculated using the method of Chou et al. [19].

brane helices is common [24]. These residues are thought to play functional roles in stabilizing monomer association (as in ion channels) or in the signal transduction process in GPCRs. Our analysis indicates that interhelical contacts in these helices mimic those of coiled-coils and other non-proline containing helices, with large hydrophobic or aromatic residues located either 3 or 4 residues apart in the sequence. In addition, the concave side of TM helix III, created by the kink at P92, has several residues strategically located near helices V and VI. These helices also have close contacts as noted in Figure 2 and in the case of helix VI, contain yet another proline residue, this time located at the opposite end of the helix (relative to P92 in helix III). The interactions between helices III and V, and III and VI, once again, follow the same structural motif. Contacts in the latter occur mainly between aromatic residues (Y83-W189, W86-Y185, and T90-W182) while contacts in the former are dominated by large hydrophobic groups (L94-I148, L97-L152).

The generality of the basic knob into hole contact type to linked helical interactions is further supported by the structure of melittin [16]. To the best of our knowledge, this is the only other structural example of residue contacts among pairs

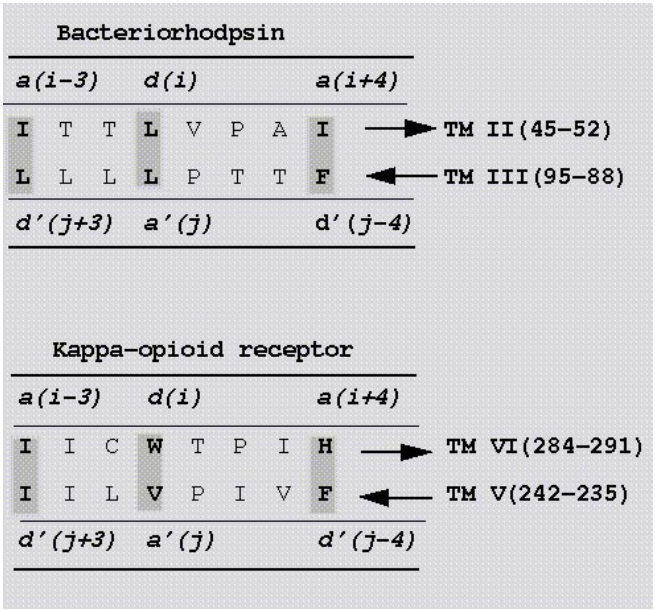


Figure 3. *Schematic of van der Waals inter-helical contacts and assignment of residues to the a-d notation (from fig. 1). An alternative numbering scheme is also given, to uniquely identify each residues (see text). Interacting residues are boldface and shaded. Arrows indicate chain direction. Schematic of van der Waals helical contacts between TM II and TM III of BR (above); proposed contacts between TM V and TM VI of GPCRs. The kappa-opioid receptor is given as an example (below).*

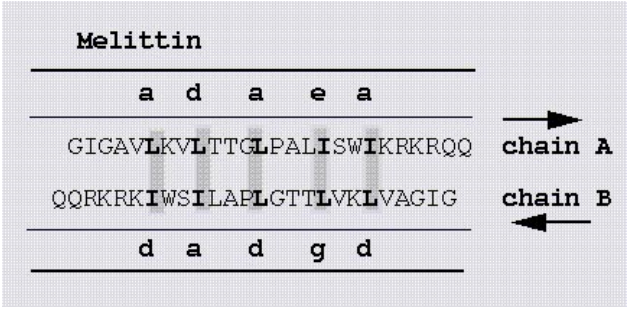


Figure 4. *Schematic of van derWaals helical contacts between chain I and chain II of the melittin dimer. Interacting residues are boldface and shaded. Arrows indicate chain direction.*

of proline-containing helices. Proline occurs at position 14 of this 26 residue helical peptide. These peptide monomer units self-assemble into tetramers in aqueous solution [16]. The crystal structure consists of a pair of helical dimers oriented in an antiparallel fashion [16]. Despite significant bending of the helices, the interhelical close contacts involve large hydrophobics at successive *a* and *d* positions, as seen in BR and other coiled-coils (with and without proline residues) (Figure 4) In addition, contacts are found between residues at positions *e* and *g* at adjacent positions to the prolines. These contacts, however, are most likely a result of local packing requirements that are altered by the close proximity of the proline-kinks in these sequences. The interhelical tilt angle of the helices in the dimer, -162 degrees, and distance of closest approach, D, are also consistent with previously reported values (as well as those given in Table 1).

Rhodopsin-like GPCRs also contain highly conserved proline residues in several TM helical sequences. These prolines formed the basis to the non-sequential alignment procedure used in a previous study to model GPCR helix packing in the beta-2-adrenergic receptor [6]. The alignment of GPCR helices V and VI to BR helices III and II, respectively, has been further extended here to include a total of 332 GPCR sequences [3]. An analysis of knob-into-hole contact positions of these sequences shows a strong correlation between residue types in BR and the GPCRs, as shown in Figure 3. Alignment of helix III of BR and GPCRV results in the matching of a highly conserved Phe residue at *d'* (j–4), BR: F95 to GPCR:F242, and a common pattern of four consecutive hydrophobic residues following proline, two of which are located at *a'* (j) and *d'*(j+3). Alignment of helix II of BR and GPCR VI shows hydrophobic residues at *a* (i–3) while positions *d* (i) and *d* (i+4) are occupied by hydrophobic residues in BR and by aromatic residues in GPCRs. Despite specific amino acid differences, the conservation of aromatic or hydrophobic residues in positions *a* and *d* involved in helical contacts suggest both pairs of helices are in part, stabilized by this network. Figure 3 illustrates the interaction scheme for the particular case of the kappa-opioid receptor.

A PERSCAN sequence analysis of GPCR helix V and VI indicates these helical contacts are most likely directed towards the interior of the receptor. The amino acid substitution profiles of these two helices shows that residues I285, T288, V236, and L240 (using the kappa-opioid sequence) are likely to be exposed to the lipid environment, while F242, V238, I235, I284, W287 and H291 face the interior of the receptor. These positions, however, are known to be oriented in opposite directions in BR helices III and II. This suggests that the relative packing of GPCR helices V and VI is also reversed with respect to the packing of helices III and II in the BR crystallographic structure. This is reflected by the PERSCAN direction vectors shown in Figure 5. These vectors indicate GPCR helices V and VI should be re-oriented 180 degrees with respect to BR helices III and II in the overall packing of the TM domain. Such a manipulation would place both helix pairs in a counter clockwise rotation within the TM domain, in concert with results derived from site directed mutagenesis studies and biophysical studies of zinc binding mutants [7,8].

The re-orientation of GPCR helices VI and V proposed also reverses the position of the proline residues with respect to the lipid environment. That is, the convex side of helix V, which has the more pronounced kink, now faces the interior of the receptor while in BR, this structural feature of helix III faces the lipid. A previous study of prolines in transmembrane helices has shown, however, that the orientation of the convex side in BR helix III is unique [24]. Most proline containing helices prefer a convex-in orientation. The reversal of this face in BR helix III was attributed to the position of polar and charged residues in the sequence. These residues are absent from GPCR helix V, which is considerably more hydrophobic than BR helix III supporting the more common packing arrangement proposed here.

Extension to non-rhodopsin-like sequences

The occurrence of highly conserved proline residues within the TM domain of GPCRs is not unique to the rhodopsin-like family. These residues can be found in the interior of transmembrane helices in the calcitonin-like (family B), the metabotrobic-like (family C), the pheromone-like (family D) and the cAMP (family E) receptor families [3]. Although the precise position of these prolines is not conserved across families, the residues follow a similar pattern or distribution within the TM domain, suggesting a possible structural or functional connection. As in the rhodopsin- like family, conserved prolines are found only in TM helices IV to VII and always occur in consecutive helices, as shown in Figure 6. The relative depth within the TM domain, however, differs from family to family. The closest similarities are found for GPCRs in families D and E (see Table I) where prolines are located approximately half-way into the TM domain in neighboring helices.

An alignment of the proline residues in BR helices II and III with those of helices IV and V in family D and VI and VII

in family E results in the interaction scheme shown in Figure 7. As in family A, the residues identified are mostly large hydrophobics or aromatics and obey the general knob-into-holes packing motif outlined above. Additional similarities can be found in the results of PERSCAN analyses of these helix sequences. The direction vectors derived from lipid facing substitution tables place these residues in close proximity along neighboring faces of the helix pair. Based on these vectors, and the contact scheme proposed, the helices once again follow a counter clockwise arrangement (as viewed from the extracellular side) within the TM domain. It should be emphasized, however, that this arrangement is highly tentative due to the limited sequence information available for GPCR families D and E (Figure 7).

Conclusion

An analysis of helix contacts in the structure of BR has been presented and extended to study potential packing arrangements in the rhodopsin-like GPCRs. In the past, commonalities between the structural features of these proteins have been noted in the same number of transmembrane helices, the two layer design, and a pair of proline-contain-

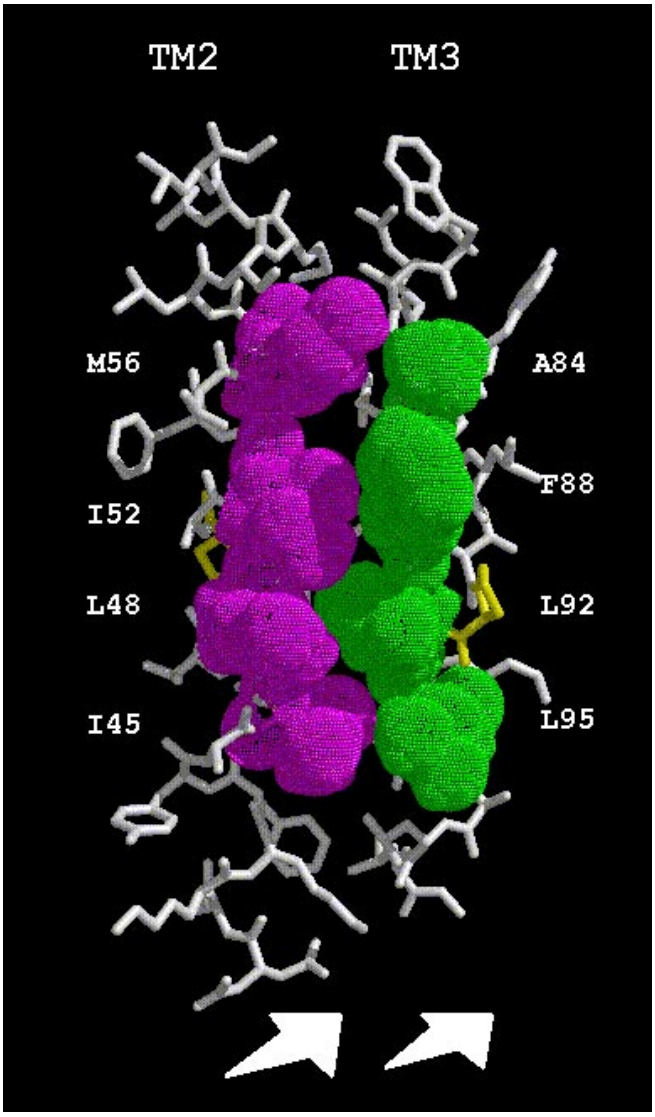
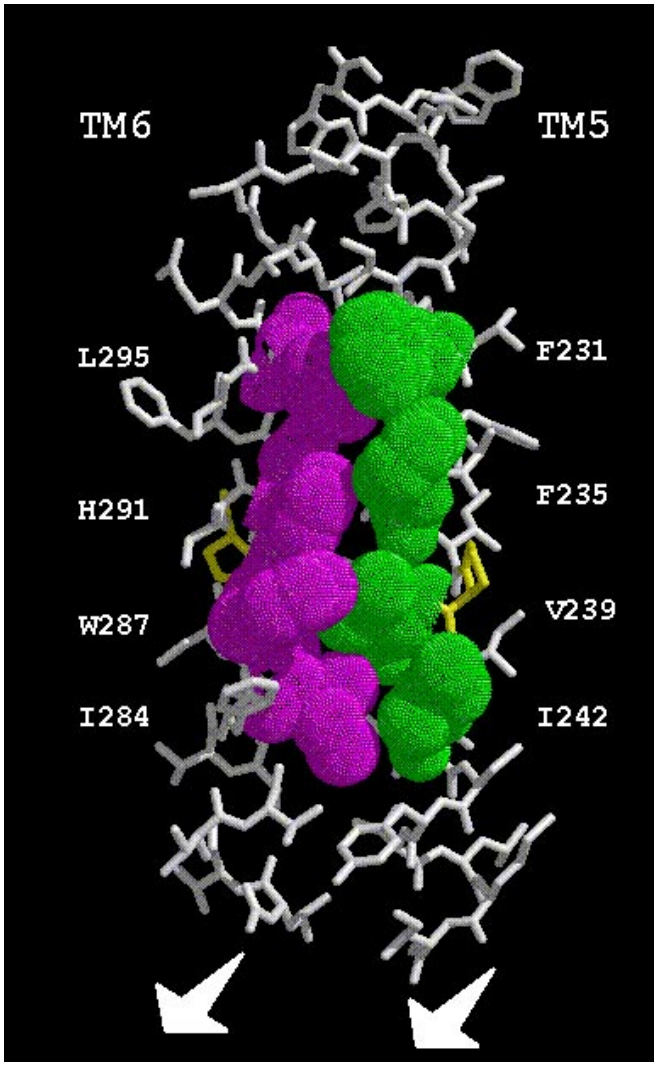


Figure 5 (a). *Schematic 3-D model of TM II and TM III of BR, illustrating van der Waals contacts between the two helices. Residue names and numbers are indicated. Arrows point to the interior of the seven helixbundle.*



(b). *Schematic 3-D model of TM V and TM VI of the kappa-opioid receptor illustrating proposed van der Waals contacts between the two helices. Residue names and numbers are indicated. Arrows point to the interior of the seven helix bundle.*

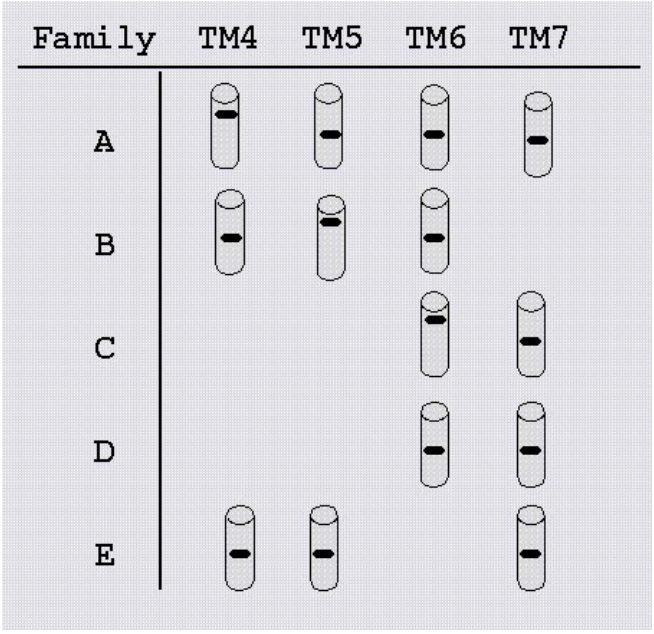


Figure 6. *Distribution of highly conserved prolines in transmembrane helices in the five families of GPCRs. Helices are shown as cylinders with black lines indicating the approximate position of Pro along each helix.*

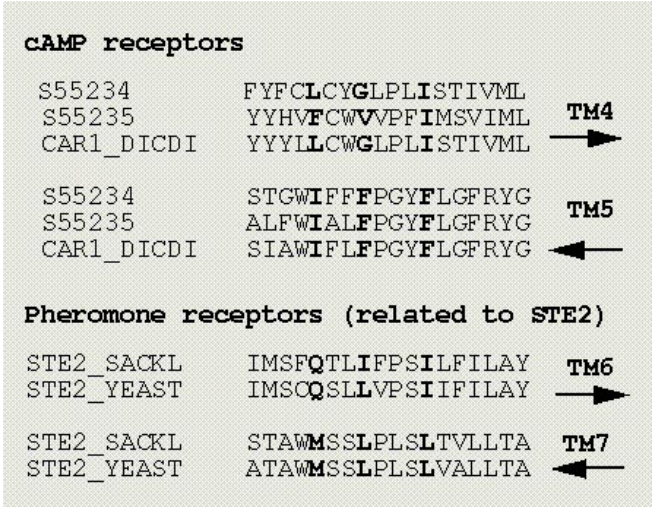


Figure 7. *Proposed interhelical contact for helices TM IV and TM V of the cAMP receptors and for helices TM VI and TM VII of the pheromone receptors. Interacting residues are boldface and shaded. Arrows indicate chain direction. Sequences are taken from the OWL database [3].*

ing helices oriented perpendicular to the plane of the membrane. The results presented here describe further similarity in the pattern of amino acid residues involved in helix contacts flanking highly conserved prolines in these proteins which is consistent with a knob-into-hole mode of interaction. Although this scheme is seen in helical contacts in globular proteins and photosynthetic reaction centers, examples of residue contacts among pairs of proline containing helices are rare. Besides BR, there is only one other structure in the Brookhaven Protein Databank that has this unique structural arrangement, melittin. Our analysis has shown this peptide structure also displays knob-in-hole contacts that may, in fact, help stabilize dimerization. There is some experimental evidence that these contacts play a similar role in BR and the GPCRs. Mutations of Pro to Ala in helix V and VI of the muscarinic M3 receptor has been shown to result in lower expression levels and reduced affinity for the endogenous ligand [25]. In BR, Pro to Ala mutations in helices II and III is known to slow regeneration rates of the chromophore [26]. Given the increased rigidity of the backbone near proline and the inherent bend of the helices that favors knob in hole packing of side chains, it is reasonable to conclude these interactions are in some way involved in stabilizing the structure. Although hydrophobic interactions of this type are well known forces in protein folding, the folding mechanism for receptors is not yet understood. It has been previously suggested that BR and other related proteins fold through a two stage mechanism in which the initial step involves secondary structure pre-assembly [27]. Our analysis suggests that some tertiary interactions may also form at this stage further directing the structural organization.

The analysis performed has also provided insight to the overall helix packing arrangements in the rhodopsin-like GPCRs. Although the results are limited, the GPCR model building experiments described have allowed the overall chirality or handedness of the receptor to be assigned. Although different schemes have been proposed in the past [5,28], the analysis presented here supports a sequential counter clockwise arrangement (as viewed from the extracellular side) similar to that of BR. This issue has recently been addressed, although indirectly, using site directed mutagenesis studies. Mutational analyses of hybrid muscarinic receptors have identified a keycontact between residues in helices I and VII that can only be satisfied based on a counter clockwise arrangement. Additional support for this packing can be found in the zinc ion binding properties of a mutant kappa-opioid receptor [7]. Point mutations to His of three residues located near the extracellular end of helices V and VI resulted in the creation of zinc ion binding site revealing the relative proximity of these histidines. The structure obtained here is consistent with these results (Figure 5). Substitution of K227, D223, and A298 of the kappa-opioid receptor with His places the three imidazole rings in a relative position suitable for ion chelation [7].

Finally, our analysis of cAMP and pheromone-like sequences has revealed commonalities with BR may extend beyond the rhodopsin-like GPCRs. Although the sequence information is limited, the rough alignments performed indicate a similar knob-into-holes packing motif is possible among pairs of proline-containing helices in these GPCRs.

The depth of the prolines is also consistent with those found within the TM domains of BR and the rhodopsin-like GPCRs. While no function has yet been linked with the occurrence of these highly conserved prolines within the TM domain, the similarities suggest these residues may play a common structural or functional role in BR and the GPCRs (possibly in structure pre-assembly as mentioned above). The helix contacts predicted here may also be important in this regard and may ultimately provide clues to the overall function of the rare pairing of prolines in the TM domains of GPCRs.

Acknowledgement. This work has been supported by NIH/ NIDA grant R55DA/0D08949. M. G. Paterlini acknowledges support of NIDA training grant T32DA07234.

References

1. Schertler, G.F.X.; Villa, C.; Henderson, R. *Nature* **1993**, *362*, 770.
2. Baldwin, J. M. *EMBO J.* **1993**, *12*, 1693.
3. Attwood, T. K.; Findlay, J. B. C. *Prot. Engng.* **1994**, *7*, 195.
4. Teeter, M.M.; Froimowitz, M.; Stec, B.; DuRand, C.J. *J. Med. Chem.* **1994**, *37*, 2874 .
5. Pardo, L.; Ballesteros, J.A.; Osman, T.; Weinstein, H. *Proc. Natl. Acad. Sci. USA.* **1992**, *89*, 4009.
6. Metzger, T.G.; Paterlini, M.G.; Portoghese, P.S.; Ferguson, D.M. *J. Comp. Inf. Comput. Sci.* **1996**, *36*, 857.
7. Thirsrup, K.; Elling, C. E.; Hjiorth, S. A.;, Schwartz, T. W. *J Mol.Bio.* **1996**, *271*, 7875.
8. Liu, J.; Schoneberg, T.; van Rhee, M.; Wess, *J. Biol. Chem.* **1995**, *270*, 19532.
9. Crick, F.H.C. *Acta Cryst.* **1953**, *6*, 689.
10. Cohen, C.; Parry, A.D. *TIBS* **1986**, *11*, 245.
11. McLachlan, A. D.; Stewart, M. *J. Mol. Bio.* **1975**, *98*, 293.
12. Chothia, C.; Levitt, M.; Richardson, D. *J. Mol. Bio.* **1981**, *145*, 215.
13. Walther, D.; Eisenhaber, F.; Argos, P. *J. Mol. Bio.* **1996**, *55*, 536.
14. Rees, D.C.; Chirino, A.J.; Kim, K.-H.; Komiya, H. In:"Membrane protein structure." S H. White editor. Oxford University Press, New York. 1994, 1.
15. Henderson R.; Baldwin, J.M.; Ceska, T.A.; Zemlin, F.; Beckman, E.; Dowing, K.H. *J. Mol. Bio.* **1990**, *213*, 899
16. Terwilliger, T.C.; Eisenberg, D. *J. Biol. Chem.* **1982**, *257*, 6010.
17. Bernstein, F.C.; Koetzle, T.F.; Williams, G.J.B.; Meyer, E.F. Jr.; Brice, M.D.; Rodgers, J.R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. *J. Mol. Biol.* **1977**, *112*, 535.
18. Ferrin, T.E.; Huang, C.C.; Jarvis, L.E.; Langridge, R. *J. Mol. Graphics* **1988**, *6*, 13.
19. Chou, K.C., Nemethy, G., Scheraga, H.A. *J. Phys. Chem.* **1984**, *106*, 3161.
20. Cornell W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmayer, D. C.; Fox T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179.
21. Pearlman, D. A.; Case, D. A.; Caldwell, J. W.; Ross, W. S.; Cheatham T. E.; DeBolt S.; Ferguson , D. M.; Seibel, G.; Kollman, P. *Comp. Phys,. Comm.* **1995**, *91*, 1.
22. Donnelly, D.; Findlay, J. B. C.; Blundell, T. L. *Receptors and Channels* **1994**, *2*, 61.
23. Lemmon, M. A.W, Engelman, D. M. Q. *Rev.Biophys.* **1994**, *27*, 157.
24. von Heijne. *J. Mol. Bio.* **1991**, *218*, 499.
25. Wess, J.; Nanavati, S.; Vogel, Z.; Maggio, R.. *EMBO J.* **1993**, *12*, 331.
26. Mogi, T.; Stern, L. J.; Chao, B. H.; Khorana, H. G. *J. Biol. Chem.* **1989**, *264*, 14192.
27. Popot, J-L. *Curr. Opi. Struct. Bio.* **1993**, *3*, 532.
28. Maloneyhuss, K.; Lybrand, T. P. *J. Mol. Bio.* **1992**, *225*, 859.