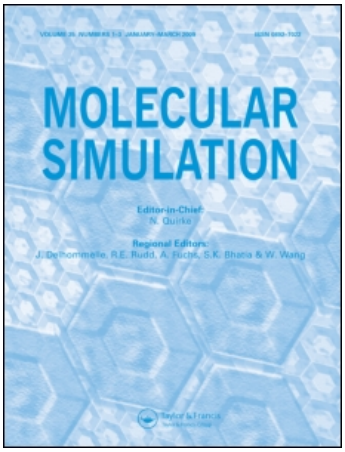

Molecular Simulation
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713644482

Comparison of the transmembrane helices of bovine rhodopsin in the crystal structure and the C α template based on cryo-electron microscopy maps and sequence analysis of the G protein-coupled receptors

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Online publication date: 26 October 2010

To cite this Article Dastmalchi, Siavoush , Kobus, Felix J. , Iismaa, Tiina P. , Morris, Michael B. and Church, W. Bret(2002) 'Comparison of the transmembrane helices of bovine rhodopsin in the crystal structure and the C α template based on cryo-electron microscopy maps and sequence analysis of the G protein-coupled receptors', Molecular Simulation, 28: 8, 845 — 851
To link to this Article: DOI: 10.1080/0892702021000002520
URL: http://dx.doi.org/10.1080/0892702021000002520

Molecular Simulation, 2002 Vol. 28 (8–9), pp. 845–851

COMPARISON OF THE TRANSMEMBRANE HELICES OF BOVINE RHODOPSIN IN THE CRYSTAL STRUCTURE AND THE C^{α} TEMPLATE BASED ON CRYO-ELECTRON MICROSCOPY MAPS AND SEQUENCE ANALYSIS OF THE G PROTEIN-COUPLED RECEPTORS

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(Received February 2001; in final form November 2001)

G protein-coupled receptors (GPCR) are activated by a diverse array of extracellular signals, ranging from light to polypeptide molecules. The receptors propagate these signals intracellularly using G protein secondary messenger pathways. A common feature in the architecture of these receptors is their seven transmembrane domains. The first crystal structure of a GPCR, bovine rhodopsin, has recently been solved at 2.8 Å. We compared the seven membrane-spanning helices (TMH) from the crystal structure of bovine rhodopsin with those from the low-resolution model of bovine rhodopsin based on the cryo-electron microscopy structure of frog rhodopsin developed by Dr Joyce Baldwin. The model developed by Baldwin used a consensus sequence approach to predict the rotational position of each helix with respect to the other six helices. Superposition of the entire helix bundle of the Baldwin model with the crystal structure gave a RMS difference (RMSD) of 3.2 Å for the 198 C^{α} atoms which suggests a high level of similarity in the arrangement of the helices. Except for TMH IV (RMSD of 4.0 Å), the position of corresponding helices within the helix bundle overlapped well. The superposition of individual helices showed that the RMSD values over 3 Å in the global superposition were largely due to one or more of the following: (i) differences in the unraveling and kinks for these helices, (ii) translation of TMH perpendicular to the membrane and (iii) rotation of helices up to 31°,

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ISSN 0892-7022
DOI: 10.1080/0892702021000002520

except for TMH IV in which an additional contribution to the RMSD came from the aforementioned observation. As other crystal structures of GPCRs become available, a comparison with the Baldwin consensus model may reveal larger differences than those observed here.

Keywords: G protein coupled receptors; rhodopsin; structure comparison

INTRODUCTION

The rhodopsin-like G protein-coupled receptors (GPCRs) form a unique family of hundreds of proteins, which transduce chemical and optical signals through the cytoplasmic membrane by activating intracellular G proteins [1]. Activation of G proteins in turn triggers a wide range of biological events in the cell. A detailed structural knowledge of GPCRs is of interest because they are prime pharmacological targets for the development of new therapeutic agents. Structural information on any member of the GPCR family might therefore be used for rational drug design. GPCRs share a conserved transmembrane structure and before the X-ray structure of bovine rhodopsin became available [2], the electron microscopy of several rhodopsins had clearly shown that these receptors consist of seven transmembrane α-helices [3,4]. Rhodopsin is a GPCR responsible for absorption of photons in retinal rod photoreceptor cells and it has attracted much attention in terms of the elucidation of both how it performs its physiological role and its structure. Rhodopsin is composed of the protein opsin covalently linked to 11-*cis*-retinal through Lys^{296}.

Recently the crystal structure of bovine rhodopsin was solved by Palczewski *et al.* [2] at a resolution of 2.8 Å and represents the first high-resolution crystal structure of a GPCR. There is great potential for this crystal structure to be used as a template for modeling other GPCRs, but such work assumes that rhodopsin represents a consensus template for the structure of other GPCRs. The validity of this assumption is yet to be proven.

Prior to the publication of this structure, Baldwin *et al.* [4] proposed a model for the alpha-carbon (C^{α}) positions in the seven transmembrane helices in the rhodopsin family of GPCRs. Structural information, including the location of the seven helical segments, their lengths and relative orientations, had been deduced from the analysis of 493 sequences of GPCRs and was incorporated into the model. The three-dimensional electron density map of frog rhodopsin [3] determined by electron cryo-microscopy, with an effective resolution of 7.2 Å in the membrane plane and 16.5 Å normal to it, was used to assist the positioning of the helices (two kinked and five straight ideal helices) in the model. The model included the membrane-spanning portions of the helices as well as continuations

of the helices beyond the membrane on both sides. In this work, we refer to transmembrane helices as these extended helices. The usefulness of this model, known as the Baldwin model, is also based on it representing a consensus template for the structure of other GPCRs. Here we present a comparison between the Baldwin model and the X-ray structure of bovine rhodopsin.

COMPARING THE CRYSTALLOGRAPHIC STRUCTURE AND THE BALDWIN MODEL FOR RHODOPSIN

Methods

Calculations were performed on a Silicon Graphics O2 workstation (SGI, Mountain View, CA, USA) using the InsightII molecular modelling package (v98.0, Molecular Simulations, San Diego). The Baldwin model of bovine rhodopsin was obtained directly from Dr Joyce Baldwin [4]. It consists only of the C^{α} atoms in transmembrane helices. The crystallographic structure of rhodopsin was obtained from the Protein Data Bank at the Research Collaboratory for Structural Bioinformatics [5]. There are two structures in the asymmetric unit for rhodopsin in the PDB file (1f88). Both these structures, A and B, were compared with the Baldwin model. Comparison was made only for regions of structure common to both the Baldwin model and each of A and B crystallographic structures. Briefly, the C^{α} atoms of the model and each of the structures A and B were superimposed using the criterion that the root mean square of distance (RMSD) be minimized. RMSD between corresponding residues was then calculated for each helix after superposition of the entire helix bundle (global) and in separate helix superpositions (individual helix).

In addition, the rotational positioning was compared in the corresponding helices in the Baldwin model versus the X-ray structures after superposition of the C^{α} of the individual helices. The angle of rotation was calculated in the plane of the graphics screen between the geometric centers of the two corresponding helices about the superimposed helix axis with the helix axis positioned perpendicular to the screen (*xy* plane). As an alternative to the RMSD comparison, contact area difference (CAD) [6] between the two rhodopsin molecules in the asymmetric unit of the crystal was calculated using the ICMlite program (v 2.8 2000, MolSoft L.L.C., La Jolla, CA). Secondary structure conformations were identified using the Kabsch–Sander method [7].

A relative translation of the corresponding helices between structure A and the Baldwin model was calculated along the helix axes. First, the geometric center for each helix was determined and then the X-ray structure and the Baldwin

model were superimposed globally and positioned on the graphic screen so that helix bundle axes were perpendicular to the *xz* plane. Finally, the relative distances of the geometric centers of corresponding helices on the *y* axis of the screen were measured as an indicator of the relative translation of the helices.

RESULTS AND DISCUSSION

Using the transmembrane definitions postulated by Baldwin *et al*., [4], global superposition of the helix bundles of the Baldwin model of bovine rhodopsin with structure A from the X-ray study [2] gave on RMSD value of 3.2 Å for 198 C^{α} atom pairs. Using structure B, the RMSD value was 3.1 Å for 191 C^{α} atom pairs. (Unless otherwise stated, values in parentheses below represent results for structure B.) The RMSD between structures A and B were 0.6 Å (C^{α}) for 305 atom pairs and 1.2 Å for all non-hydrogen polypeptide atoms. The CAD value, which may by a more sensitive estimate of side-chain conformational differences, was 11.2%, which is unusually large in comparison to other crystal structures [6].

The low RMSD value of 3.2 Å suggests strong similarity between the crystal structure and the Baldwin model in the packing of the helices within the helix bundle. However, several residues defined by Baldwin as being part of a helix (Arg^{69} in TMH II, Lys^{141} and Pro^{142} in TMH III, Trp^{175} in TMH IV, Thr^{229}-Ala^{233} in TMH V, and Asn^{310}-Lys^{311} in TMH VII) were not in the helical regions in the crystal structure (Fig. 1). The exclusion of these residues from the calculation of the global superposition of the helix bundles reduced the RMSD value to 2.9 Å for the 187 remaining C^{α} pairs (3.0 Å for 185 C^{α} pairs). Except for TMH IV with an RMSD of 4.0 Å (5.0 Å), the positions of corresponding helices within the helix bundle overlapped well in the global superposition. TMH V also had a large RMSD value of 4.1 Å but exclusion of residues Thr^{229}-Ala^{233} decreased the RMSD value to 3.1 Å (Table I).

The superposition of individual helices showed that TMH I, II, III, IV and VI all had relatively small RMSDs of $\leq$ 2.1 Å. The small value for TMH IV of 1.9 Å, compared to its large value in global superposition, suggests that the Baldwin model displaces the helix from the bundle compared to its position in the crystal structure. On the other hand, the RMSDs for TMH V and VII of 3.5 and 2.5 Å (2.6 Å), respectively, suggest specific differences in unraveling and kinks for these helices in the X-ray structure compared to the Baldwin model.

Two other factors contributing to the global RMSDs were relative rotation and translation. Rotations were calculated as whole-helix parameters, with values ranging from -29 ($-31°$) to $+28$ ($+27°$) (Table I). Translational components

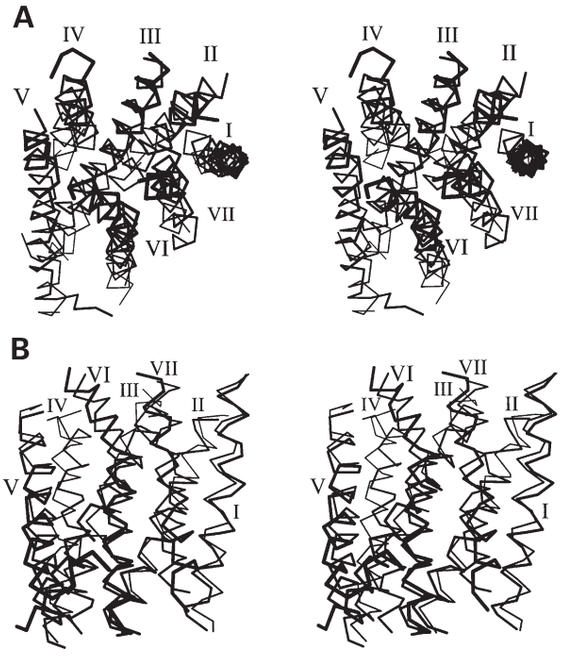

FIGURE 1 Stereoscopic view of the superimposed C^{α} models of the crystal structure (dark lines) and the Baldwin model (light lines) as viewed from the extracellular side (A), and a 90° rotation so the view is from the side (B). Figure produced with MOLSCRIPT [8].

were measured up to ~1.7 Å, which occurred in TMH IV (down in the Baldwin model relative to the crystal structure), V (up) and VI (down).

We have also examined the non-idealities in the helices of the crystal structure in terms of (i) unwinding and (ii) a change in helix axis introduced at a single point. In these comparisons, no helix component was smaller than 5 residues. For TMH II, IV, V, VI and VII, the RMSDs for the individual helical components (i.e. each of the 2 components in each of these helices) reduced to ≤ 1.3 Å. A value of 1.3 Å was also obtained for the 27-residue TMH I and for the 14-residue N-terminal region of TMH VII in which non-ideal α-helix was present in the crystal structure. The Baldwin model [4] predicted changes in helix axis because of a kink in TMH V and VI, though in the crystal structure of TMH V the kink also includes unwinding of the helix associated with Pro^{215}. The model did not predict the kinks in TMH II, IV and VII. The kinks in TMH IV and VII are associated with prolines (the Pro^{170}-Pro^{171} and Pro^{303}, respectively). The kink in TMH II occurs at Gly^{89}, is not proline induced, and includes a large component of unwinding.

TABLE I Comparison between the Baldwin model [4] and crystallographic structures of bovine rhodopsin [2]

		RMSD (Å)		
TMH	Residues	Global	Individual helix	Relative rotation (degree)*
I	Ser^{38}-Gln^{64}	2.3 (2.5)	1.3 (1.3)	−27 (−27)
II	Arg^{69}-Leu^{95}	3.3 (3.0)	2.1 (2.1)	+25 (+22)
	Thr^{70}-Leu^{95}	3.4 (3.5)	1.8 (1.8)	+27 (+25)
III	Thr^{108}-Pro^{142}	2.7 (2.6)	1.7 (1.8)	+26 (+25)
	Thr^{108}-Cys^{140}	2.2 (2.2)	1.2 (1.1)	+28 (+27)
IV	Asn^{151}-Trp^{175}	4.0 (5.1)	1.9 (2.0)	−12 (−10)
	Asn^{151}-Gly^{174}	3.9 (3.8)	1.5 (1.6)	−15 (−13)
V	Val^{204}-Ala^{233}†	4.1	3.5	+15
	Val^{204}-Phe^{228}†	3.1	2.0	+23
	Val^{204}-Leu^{226}	3.1 (2.7)	2.0 (2.1)	+26 (+26)
VI	Lys^{245}-Tyr^{274}	2.5 (3.4)	1.1 (1.1)	+6 (+5)
VII	Met^{288}-Lys^{311}	3.2 (3.2)	2.5 (2.6)	−29 (−31)
	Met^{288}-Met^{309}	3.2 (3.2)	2.3 (2.4)	−25 (−26)

Figures are for the comparison between the Baldwin model and structure A, and the Baldwin model and structure B (parentheses) of rhodopsin in the crystallographic structure.
* Positive values represent the clockwise rotation of the helix in the Baldwin model relative to the crystal structure as viewed from the extracellular side.
† Residues 227–233 were not available in structure B.

We conclude that the Baldwin model [4] and the crystal structure [2] show a high level of similarity. The Baldwin model predicted helix kinking in TMH V and VI and predicted the relative rotation of helices quite well (within ± 31°). The Baldwin model did not predict the mid-helix unwinding in TMH II and V observed in the crystal structure or changes in helix direction within TMH II, IV and VII. The structural differences we have observed appear relatively subtle, but may be sufficient to affect structure–activity relationships significantly. Furthermore, if parameters such as hydrophobicity and atomic packing are the driving forces in membrane protein folding, a comparison of the crystal structures of other GPCRs with their models built based on the Baldwin consensus model may reveal larger differences than those observed in this comparison.

Acknowledgements

The authors would like to thank Dr J.M. Baldwin for the model coordinates and colleagues at the Garvan Institute of Medical Research for valuable discussions and encouragement. This work was supported by a scholarship from the Iranian Ministry of Health and Medical Education to SD, Australian Postgraduate Award to FJK, NHMRC of Australia funding for TPI and Australian Research Council and Sydney University Sesqui R&D grants to MBM and WBC.

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