African Journal of Biotechnology Vol. 11(2), pp. 466-473, 5 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.167
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Comparative homology modeling of human rhodopsin with several templates of bovine rhodopsin

Shagufta Kanwal[1], Sumaira Nishat[1], Muhammad Irfan Khan[1], Shaukat Iqbal Malik[2*] and Khalid Akhtar[3]

[1]Department of Bioinformatics and Biotechnology, International Islamic University, Sector H-10 Islamabad, Pakistan. Email: shagufta.kanwal@iiu.edu.pk.
[2]Bioinformatics and Molecular BiologyResearch Center (BMBRC), Mohammad Ali Jinnah University, Zone V, Islamabad, Pakistan. Email: drsimalik@jinnah.edu.pk.
[3]SMME, National University of Science and Technology (NUST) H-12 Islamabad, Pakistan.

Accepted 30 September, 2011

Rhodopsin is a visual pigment, which belongs to G-coupled protein receptor (GPCR) family, present in rod cells of retina. The molecular structure of rhodopsin has been studied by cryo-electron microscopic, Nuclear Magnetic Resonance (NMR) and X-ray crystallographic techniques in bovine. A humble effort has been made to identify and remove the loopholes in the existing structure (1EDS) and model the 3D structure of rhodopsin to its entire length and loops. Our studies describe the molecular structure of human rhodopsin based on homology search among seven selected bovine templates (140 models created), which were then analyzed completely through model evaluation softwares to build one complete structure of human rhodopsin. This structure will prove to be helpful in studying different biological and chemical reactions occurring in visual process, as well as hold a promising future for agonist/drug development.

Key words: Homology modeling, human rhodopsin, bovine templates, sequence alignment, model building, energy profiles.

INTRODUCTION

Rhodopsin is a visual pigment belonging to G-coupled protein receptor family (GPCR). Henderson et al. (1990) described that it has seven transmembrane α-helix structures containing seven loops and present in rod cells of retina. The seven transmembrane helices are arranged as seen in the cryo-electron microscopy studies, with the same topology as found in bacteriorhodopsin. The existing structure of rhodopsin exists in swissprot database (http://www.expasy.org/uniprot/P08100) and the structure is available at (http://www.rcsb.org) with the pdb id (1EDS). The name of this structure is "Solution Structure of Intradiskal Loop 1 of Bovine Rhodopsin (Rhodopsin Residues 92-123)". This shows sequence similarity of bovine rhodopsin with one loop of human rhodopsin protein, but this information is not sufficient for structural and functional analysis of human rhodopsin.

*Corresponding author. E-mail: drsimalik@jinnah.edu.pk.

Nathans and Hogness (1984) isolated and completely sequenced the gene encoding human rhodopsin and found many conserved intronic regions as well as perfectly conserved amino acid regions which revealed that it is 93.4% homologous to that of bovine rhodopsin. Yokoyama and Yokoyama (1989) also compared the genes (DNA sequences) encoding human, bovine, and Drosophila rhodopsins and a phylogenetic tree was constructed. This evolutionary tree shows that the common ancestor of the visual color pigment genes diverged first from that of the rhodopsin genes (Figure 1).

According to Heymann and Subramaniam (1997), bovine rhodopsin is approximately 95% homologous to human rhodopsin; the results with bovine rhodopsin are likely to be fully relevant to human rhodopsin. However, Blackshaw and Snyder (1999) studied dendograms of GPCRs by clustalW analysis, which are very clear and close evidences of molecular evolution of human rhodopsin and bovine rhodopsin. These results were in close agreement to the phylogenetic analysis of

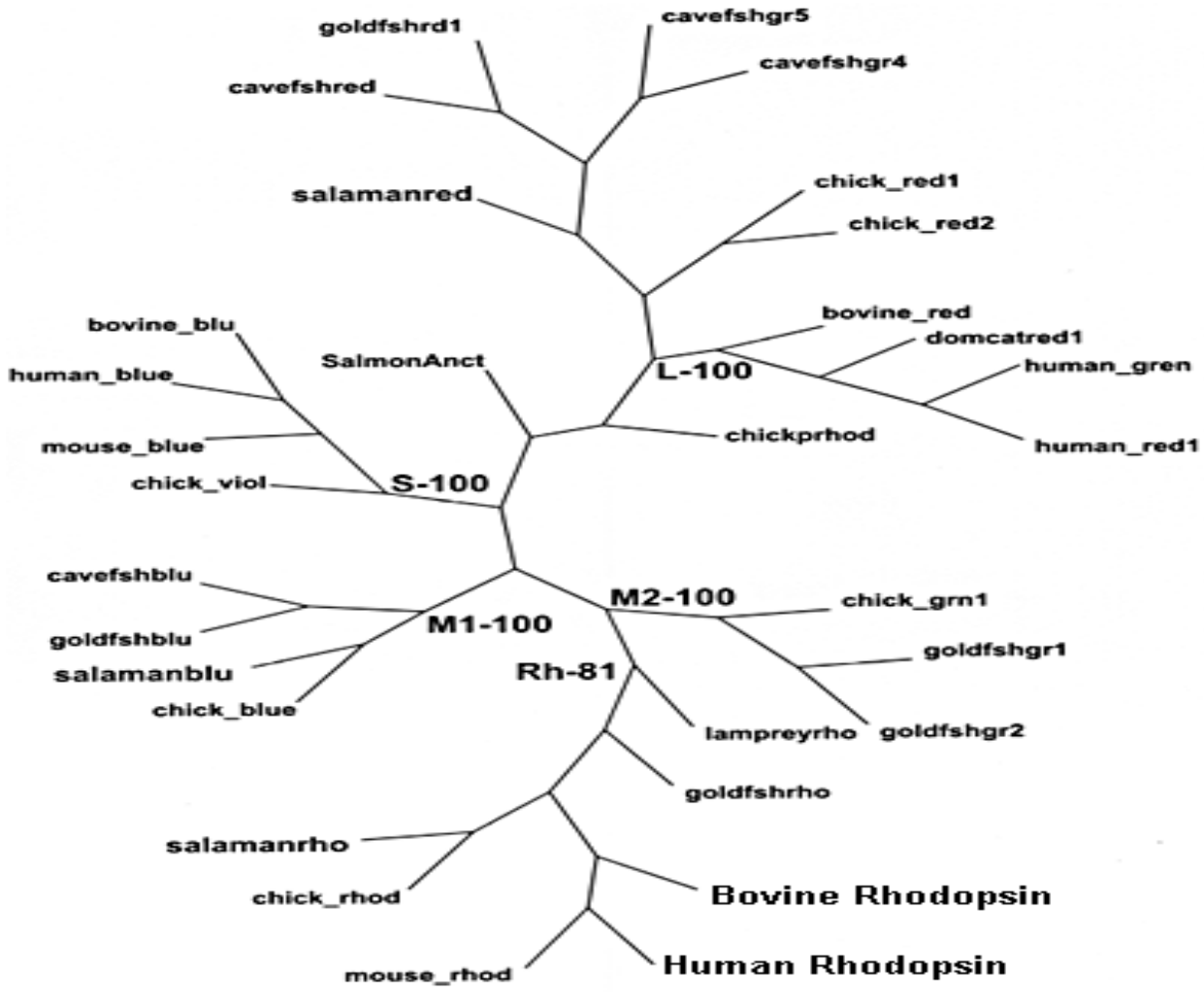


Figure 1. The putative secondary structure of these visual pigment based on the sequence similarity to bovine rhodopsin.

rhodopsin and opsin proteins in different species done by Xu et al. (1998). This signifies the need for further investigation on structure and function of human ' rhodopsin protein in order to update the existing protein databases for future research. Structural information often greatly enhances our understanding of how proteins function and how they interact with each other or it can, for example, explain antigenic behavior, DNA binding specificity, etc. X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy are the only ways to obtain detailed structural information.

Unfortunately, these techniques involve technical procedures and many proteins fail to crystallize at all and/or cannot be obtained or dissolved in large enough quantities for NMR measurements. The size of the protein is also a limiting factor for NMR. In the absence of experimental data, model building on the basis of the known three-dimensional structure of a homologous protein is at present the only reliable method to obtain structural information. Homology modeling refers to model building of a protein amino acid sequence query called "Target" which is comparatively modeled on the structure of homologous protein to predict the three dimensional (3D) structure and function of the target. It is one of the rapidly growing 3D structure prediction techniques for protein. Earlier work on rhodopsin had revealed the organization of this protein at low resolution 7.5 Å in the plane of the membrane and 16.5 Å resolution perpendiculars to the membrane. These studies on 3D structure predicted only the location of the seven rods as seven helices. (Schertler et al., 1995; Unger et al., 1995; Handerson et al., 1990; Unger et al., 1997; Schertler et al., 1999; Schertler et al., 2000). First, Schertler et al. (1993) observed seven transmembrane helices in the structural core of GPCRs in electron microscopic studies of two-dimensional crystals of bovine rhodopsin. In 1997, a low-resolution view of the helices was obtained from cryo-electron microscopic studies of two-dimensional crystals of frog rhodopsin (Unger et al., 1997).

These structures can be predicted by computational

means especially for GPCRs, as other membrane proteins, are notoriously difficult to crystallize (Filmore D, 2004). The first GPCR crystal structure was obtained by Okada et al. (2000) at high atomic resolution 2.8 and published by Palczewski et al. (2000) as inactive conformation of bovine rhodopsin (1F88, PDB). The second crystal structure of bovine rhodopsin (1HZX, PDB) has been developed by refining 1F88 at atomic resolution of 2.8 (Teller et al., 2001) and exists in protein databank (Berman et al., 2000). Another model 1L9H was obtained by refinement at slightly higher resolution that is 2.6 A (Okada et al., 2002). Filipek et al. (2003) had studied a comprehensive comparison among different crystal structures of bovine rhodopsin that is 1F88, 1HZX and 1L9H. However, more crystal structures of bovine rhodopsin have been uploaded in protein databank that is 1GZM at atomic resolution of 2.6 A (Li et al., 2004) and 1U19 at atomic resolution of 2.2 A (Okada et al., 2004) and two NMR structures that is 1JFP (Yeagle et al., 2001) and 1LN6 (Chio et al., 2002) which require a thorough comparison of all crystal structures to build one complete structure for human rhodopsin.

MATERIALS AND METHODS

We adopted the homology modeling approach as described by Fiser et al. (2000).

Template search and selection

Identification of best template structures is one of the critical steps in homology modeling. Templates searching were done by a web based tool Position Specific Iterative Basic Local Alignment Search Tool (PSI-BLAST) (Altschul et al., 1997). Multiple templates having most significant E-values were selected. The structures of selected templates were taken from protein data bank (Bernstein et al., 1977).

Sequence alignment

Sequence – template alignment is the second key step in homology modeling as model building depends entirely on alignment of sequence and structures. Sequence alignment was performed by using ALIGN2D command in Modeller 8V2 (Sali and Bludell, 1993). ALIGN2D implements global dynamic programming (Gotoh et al., 1982) and is preferred for aligning a sequence with structures because it tends to place gaps in a better structural context (Sali and Bludell, 1993). Sequence–template alignment was further refined by a multiple sequence alignment tool ClustalW (Thompson et al., 1994) that follows a progressive approach of multiple sequence alignment and performs global alignment.

Model building and evaluation

For the given alignment, by each template, an ensemble of 20 models were generated by Modeller 8v2 (Sali and Bludell, 1993) by applying default model building routine 'model'. The model with lowest objective function (created by each template) was selected (Sali and Bludell, 1993). The stereochemical properties were studied by Procheck (Laskowski et al., 1998) and a web based server RAMPAGE (Lovell et al., 2003). Geometric aspects of model were analyzed by WHATCHECK (Hooft et al., 1996). The analysis of non-bonded interaction between different atom types was done by ERRAT (Colovos and Yeates, 1993). Entire structure was analyzed by PROVE (Pontius et al., 1996).

RESULTS

BLASTP results of rhodopsin protein showed 93% sequence similarity with bovine rhodopsin proteins. Seven templates were selected on the basis of E value (0.0) better than threshold producing significant alignments, five X-ray crystal structures and two NMR structures, for homology model building of target protein. i) 1HZX (Crystal structure of bovine rhodopsin at 2.8 A); ii) 1F88 (Crystal structure of bovine rhodopsin at 2.8 A); iii) 1L9H (Crystal structure of bovine rhodopsin at 2.6 A); iv. 1GZM (Crystal structure of bovine rhodopsin in a trigonal crystal at 2.65 A); v) 1U19 (Crystal structure of bovine rhodopsin at 2.2 A); vi) 1JFP (NMR Structure of bovine rhodopsin, dark adapted); vii) 1LN6 (NMR Structure of bovine rhodopsin, Metarhodopsin II).

Sequence–structures alignment

The alignment results of target sequence with the templates of X-ray crystal structure generated by Modeller8v2 needed improvement as they were aligned in patches; were improved by ClustalW, so that all sequences were aligned on one chain leaving behind the second chain with dashes, showing mismatches while alignment files of NMR template structures (1JFP and 1LN6) are optimal and does not need improvement. However, in order to compare multiple bovine template sequences with human rhodopsin sequence, multiple sequence alignment was generated with ClustalW as shown in Figure 2.

Model building and evaluation

Total 140 models were created from seven bovine templates (20 from each) by Modeller8v2. One best model from each template was taken for comparative analysis. The comparative analysis of templates and targets indicate that the X-ray crystal structures of templates (1GZM, 1U19, IF88, 1L9H, 1HZX) show two chains (A and B) with many helices; but the models generated by Modeller8v2 shows one chain with seven helices. This is because target sequence had sequence similarity with one chain hence, superimposed on only one chain while the second chain was deleted by Modeller8v2 as shown in Plate 1. However, NMR structures of templates (1LN6 and 1JFP) had seven helices which were exactly superimposed on the target structure. It is quite evident from the energy graphs of dope profiles that target sequences (rhodopsin) were exactly lay on the template residues with minimum deviation which is

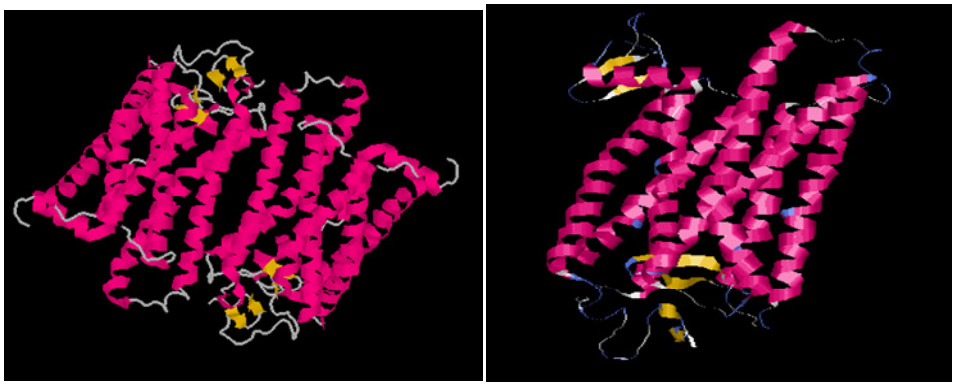
```
1JFP_A|PDBID|CHAIN|SEQUENCE     -MNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFLTLYVTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSL
1LN6_A|PDBID|CHAIN|SEQUENCE     -MNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFLTLYVTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSL
1HZX_A|PDBID|CHAIN|SEQUENCE     XMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFLTLYVTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSL
1F88_A|PDBID|CHAIN|SEQUENCE     -MNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFLTLYVTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSL
1L9H_A|PDBID|CHAIN|SEQUENCE     XMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFLTLYVTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSL
1GZM_A|PDBID|CHAIN|SEQUENCE     XMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFLTLYVTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSL
1U19_A|PDBID|CHAIN|SEQUENCE     XMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFLTLYVTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSL
ROP_SEQUENCE                    -MNGTEGPNFYVPFSNATGVVRSPFEYPQYYLAEPWQFSMLAAYMFLLIVLGFPINFLTLYVTVQHKKLRTPLNYILLNLAVADLFMVLGGFTSTLYTSL
                                 ************** ********* ********************:**************************************:****:******

1JFP_A|PDBID|CHAIN|SEQUENCE     HGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETN
1LN6_A|PDBID|CHAIN|SEQUENCE     HGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETN
1HZX_A|PDBID|CHAIN|SEQUENCE     HGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETN
1F88_A|PDBID|CHAIN|SEQUENCE     HGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETN
1L9H_A|PDBID|CHAIN|SEQUENCE     HGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETN
1GZM_A|PDBID|CHAIN|SEQUENCE     HGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETN
1U19_A|PDBID|CHAIN|SEQUENCE     HGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETN
ROP_SEQUENCE                    HGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLAGWSRYIPEGLQCSCGIDYYTLKPEVN
                                ************************************************************.*********:********** : *.*

1JFP_A|PDBID|CHAIN|SEQUENCE     NESFVIYMFVVHFIIPLIVIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSA
1LN6_A|PDBID|CHAIN|SEQUENCE     NESFVIYMFVVHFIIPLIVIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSA
1HZX_A|PDBID|CHAIN|SEQUENCE     NESFVIYMFVVHFIIPLIVIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSA
1F88_A|PDBID|CHAIN|SEQUENCE     NESFVIYMFVVHFIIPLIVIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSA
1L9H_A|PDBID|CHAIN|SEQUENCE     NESFVIYMFVVHFIIPLIVIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSA
1GZM_A|PDBID|CHAIN|SEQUENCE     NESFVIYMFVVHFIIPLIVIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSA
1U19_A|PDBID|CHAIN|SEQUENCE     NESFVIYMFVVHFIIPLIVIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSA
ROP_SEQUENCE                    NESFVIYMFVVHFTIPMIIIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWVPYASVAFYIFTHQGSNFGPIFMTIPAFFAKSAA
                                ************ **:*:*************************************************:***.**********:**************::*

1JFP_A|PDBID|CHAIN|SEQUENCE     VYNPVIYIMMNKQFRNCMVTTLCCGKNPLGDDEASTTVSKTETSQVAPA 348 93.3908 score
1LN6_A|PDBID|CHAIN|SEQUENCE     VYNPVIYIMMNKQFRNCMVTTLCCGKNPLGDDEASTTVSKTETSQVAPA 348 100.0   score
1HZX_A|PDBID|CHAIN|SEQUENCE     VYNPVIYIMMNKQFRNCMVTTLCCGKNPLGDDEASTTVSKTETSQVAPA 349 100.0   score
1F88_A|PDBID|CHAIN|SEQUENCE     VYNPVIYIMMNKQFRNCMVTTLCCGKNPLGDDEASTTVSKTETSQVAPA 348 100.0   score
1L9H_A|PDBID|CHAIN|SEQUENCE     VYNPVIYIMMNKQFRNCMVTTLCCGKNPLGDDEASTTVSKTETSQVAPA 349 100.0   score
1GZM_A|PDBID|CHAIN|SEQUENCE     VYNPVIYIMMNKQFRNCMVTTLCCGKNPLGDDEASTTVSKTETSQVAPA 349 100.0   score
1U19_A|PDBID|CHAIN|SEQUENCE     VYNPVIYIMMNKQFRNCMVTTLCCGKNPLGDDEASTTVSKTETSQVAPA 349 100.0   score
ROP_SEQUENCE                    IYNPVIYIMMNKQFRNCMLTTICCGKNPLGDDEASATVSKTETSQVAPA 348 100.0   score
                                :*****************:**:************:*************
```

Figure 2. Multiple sequence alignment of bovine rhodopsin templates (1GZM, 1U19, IF88, 1L9H, 1HZX, 1LN6 and 1JFP with target sequence of human rhodopsin ROP generated by ClustalW server (http://www.ebi.ac.uk/Tools/msa/clustalw2/).



Bovine Rho template (1GZM) Human Rho model (Rho-1GZM)

Plate 1. Comparison between bovine rhodopsin template (1GZM) and human rhodopsin protein.

ignorable. The multiple sequence alignment results show that human rhodopsin sequence has 93% sequence similarity with all bovine rhodopsin sequences taken as templates. Therefore, all bovine structures are taken as templates for structural comparisons.

Comparison of bovine rhodopsin (1GZM) template with human rhodopsin model generated by modeller

Plate 1 presents structural comparison between bovine rhodopsin template (1GZM) and human rhodopsin protein. Bovine 1GZM consisted of two chains and therefore, it has double number of helices while human rhodopsin was superimposed on one chain of 1GZM, so it had seven transmembrane helices (pink color) with beta sheets (yellow) and few turns (blue color). Depending on sequence similarity, it has been mapped on the structural constraints of 1GZM.

Evaluation of template and target through energy DOPE profile graph generated by using MATLAB

Energy profile graphs generated by MATLAB in Figure 3 show that human rhodopsin energy graph (red color) lie exactly on one chain of bovine rhodopsin template (1GZM) energy graph peaks (green color) which shows structural compatibility and similarity. Evaluation values as presented in Table 1 show that rhodopsin model formed by 1GZM template has better threshold values. Its sequence similarity with rhodopsin is 93%, and had the highest value for the favored allowed residues in Ramachandran plots is 92.8%. Its overall quality factor was good depending upon its resolution. Its Ramachandran Z-score was good and all the rest Z-scores and B-factor value were on average. So, we conclude that model formed by 1GZM template is a best possible option for target human rhodopsin.

DISCUSSION

Vriend (1998) described that the knowledge of the 3D structure is a prerequisite for the rational design of site-directed mutations in a protein and can be of great importance for the design of drugs. The field of molecular biology and bioinformatics is a great splendor of wonders in this regard. Homology modeling has opened a gateway to study detailed analysis of molecular structures. Protein 3D structures are now modeled in less time and cost with the help of bioinformatic tools instead of laborious and costly methods. The advancements in various fields like drug designing, pharmaceutics, biotechnology and nanotechnology are purely based on it.

In this study, we followed homology modeling approach for modeling a GPCR protein that is human rhodopsin depending upon five crystal structures of 1GZM, 1HZX, 1L9H, 1F88 and 1U19 and two NMR structures of 1LN6 and 1JFP. This approach is applicable when more than 40% sequence similarity between target and template exists. The templates used in this study were constructed either by X-ray crystallography or by NMR spectroscopy. Okada et al. (2000), Teller et al. (2001), Okada et al. (2002), Li et al. (2004) and Okada et al. (2004) obtained five templates that is 1F88, 1HZX, 1L9H, 1GZM, 1U19 respectively by X-ray crystallography technique while two structures that is 1JFP and 1LN6 based on NMR technique, were obtained by Yeagle et al. (2001) and Chio et al. (2002).

However, a comprehensive comparison among three crystal structures that is 1F88, 1HZX and 1L9H was done by Filipek et al. (2003), but our work is based upon comparison of seven bovine structures (total 140 models) to find the best template for homology modeling of human most of the residues are conserved and templates are

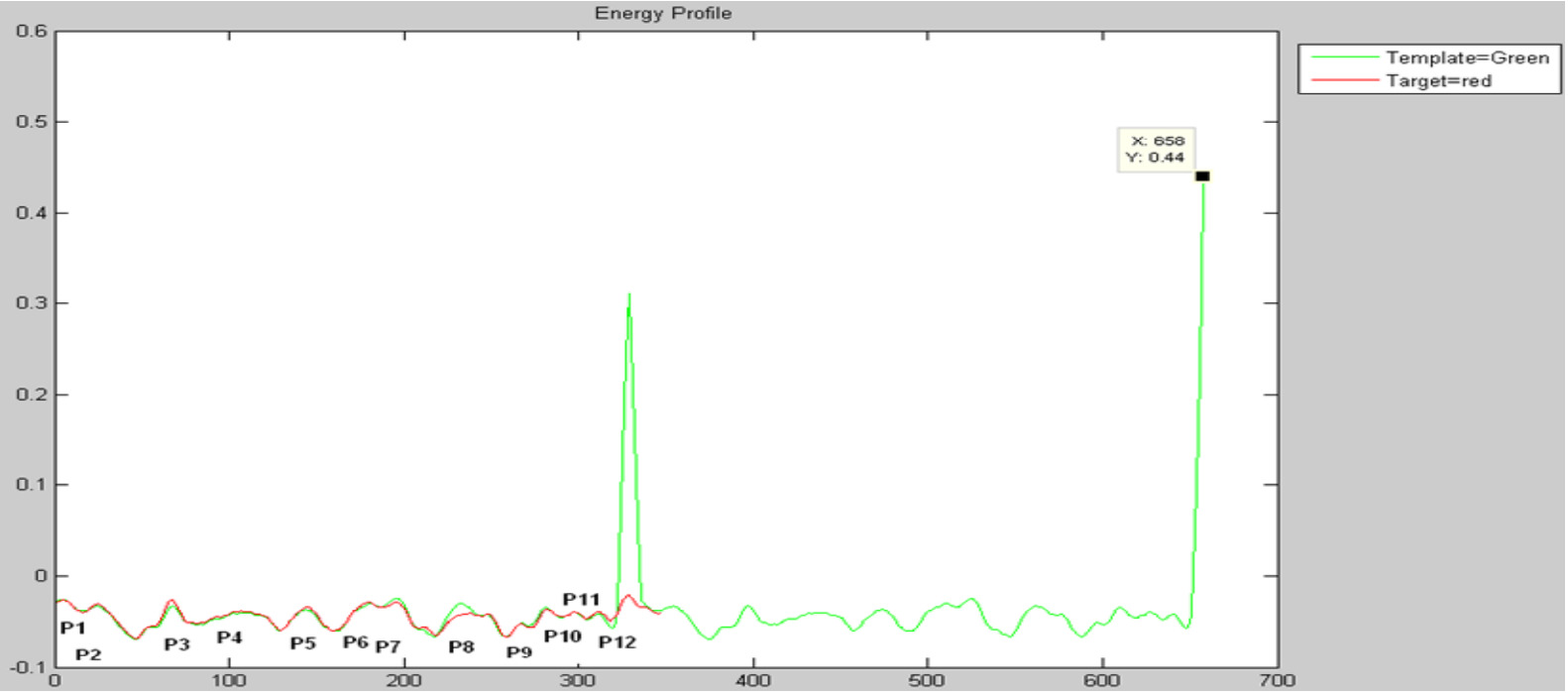


Figure 3. Dope profiles of rhodopsin model (shown in red color) and template 1GZM (shown in green color). p stands for peak numbers.

good for homology modeling. This study was in accordance to Nathans and Hogness (1984) and Heymann and Subramaniam (1997). However, the phylogenetic analysis done by Yokoyama and Yokoyama (1989), Xu et al. (1998) and Blackshaw and Snyder (1999) also verified that during evolution, rhodopsin genes diverged in different species like human and bovine but their functionality remained the same. So, bovine rhodopsin is an ortholog of human rhodopsin. After evaluation, the model formed by 1GZM template was selected as final model. As compared to the existing structure of rhodopsin that is “Solution Structure of Intradiskal Loop 1 the new model covers all the seven helices, which are required for structural and functional analysis of human rhodopsin.

This signifies the need for updating the existing

Table 1. Evaluation results on the basis of x-ray crystals and NMR templates.

	TEMPLATE	E-Value	RMS Value	DRMS Value	WHATIF Z Score	MATLAB Energy Cal Peaks	EP	Ramachandran Plot Results				Prove Plot	WHATIF REPORT RESULTS					
							OQF	MF %	AR%	DR%	Z score	Z Score RMS	RMS Z score for BL	RMS Z score for BA	CCZ score	IRMSZ-score	BCZ score	ABF
X-RAY CRYSTAL	1HZX	0.0	0.2527	0.1618	-0.180	8/14 = 57.14%	83.235	89.1	9.9	0.0	0.676	1.490	0.886	1.223	-0.664	1.206	-6.392	63.477
	1F88	0.0	0.2795	0.1616	-0.574	8/13 = 61.54%	80.826	91.8	6.9	0.0	-0.309	1.1.466	0.874	1.171	-0.285	1.209	-5.933	64.618
	1U19	0.0	0.0385	1.4946	-1.378	11/14 = 78.57%	79.118	87.8	9.2	0.7	0.167	nil	0.884	1.217	-0.319	1.225	-5.957	63.846
	1GZM	**0.0**	**0.3353**	**0.1798**	**0.877**	**11/12 = 92%**	**88.529**	**92.8**	**6.6**	**0.0**	**1.149**	**1.489**	**0.879**	**1.214**	**-0.159**	**1.194**	**-7.512**	**62.823**
NMR	1L9H	0.0	0.4188	0.2832	-0.417	5/12 = 41.66%	79.580	86.8	10.9	0.3	-0.455	nill	0.899	1.279	-0.665	1.237	-7.017	66.358
	1JFP	0.0	0.3278	0.2596	-6.938	2/10 = 20%	30.422	68.8	22.4	3.9	-6.676	nil	1.041	1.876	-3.553	1.320	-31.120	82.323
	1LN6	0.0	0.2666	0.1948	-5.805	2/8 = 25%	56.497	65.1	21.1	7.2	-5.557	nil	0.961	1.561	-3.033	1.321	-25.820	78.457

*DRMS = Distance root mean square; *EP = errate plot; *OQF = overall quality factor; *MF = most favored; *AR = allowed region; *DR = disallowed region; *BL = bond length; *BA = bond angle; *CCZ = chi-1/chi-2 correlation; *IRMZ = inside/outside root mean square; *BCZ= Backbone conformation Z score; *ABF = average B Factor.

protein databases for future research. This structure will play an important role to study protein signaling and biochemical pathways occurring in the cell. Evaluation results are in favor of human rhodopsin model with the optimal values of RMS, Z scores and B factor along with highest scores for allowed favorable residues in Ramachandran plot. Energy profiles also generate peak to peak mapping of energy graphs which shows structure compatibility and similarity.

Due to the importance of GPCRs in vast numbers of physiological processes, understanding how rhodopsin is activated, as well as other GPCRs, is one of the most fundamental problems currently unsolved in neuroscience. However, more molecular information is needed to understand how rhodopsin and other GPCRs are activated. With the progress in determination of the rhodopsin structure, Teller et al. (2001) urged for further investigations to fill the gaps in understanding how this and other GPCRs switch into the signaling state.

REFERENCES

Altschul S F, Madden T L, Schaffer A A, Zhang J, Zhang Z, Miller W, Lipman D J (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids., 25: 3389-3402; (http://www.ncbi.nlm.nih.gov/blast).

Bernstein FC, Koetzle TF, Williams GJB, Meyer J EF, Brice MD, Rodgers JR, Kennard O, Shimanouchi T, Tasumi M (1977). The Protein Data Bank: A Computer-based Archival File for Macromodel Structures. J. Mol. Biol. 112, 535-542; (http://www.rcsb.org).

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN (2000). The Protein Data Bank. Nucleic Acids Res. 28: 235–242.

Blackshaw S, Snyder S H (1999). Encephalopsin: A Novel Mammalian Extraretinal Opsin Discretely Localized in the Brain. J. Neurosci. 19: 3681-3690.

Choi G, Landin J, Galan JF, Birge RR, Albert AD, Yeagle PL (2002). Structural studies of metarhodopsin II, the activated form of the G-protein coupled receptor, rhodopsin. Biochemistry. 41: 7318-7324.

Colovos C, Yeates TO, (1993). Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci. 2: 1511-1519 (http://nihserver.mbi.ucla.edu/SAVS/)
Filmore D (2004). “It’s a GPCR family”. J. Modern Drug Discovery. 7: 11.
Filipek S, Teller DC, Palczewski K, Stenkamp R (2003). The Crystallographic Model of Rhodopsin and Its Use in Studies of Other G Protein–Coupled Receptors. Annu. Rev. Biophys. Biomol. Struct. 32: 375-397.
Fiser A, Do RK, Sali A (2000): Modeling of loops in protein structures. Protein Sci. 9:1753-1773.
Gotoh O (1982). An improved algorithm for matching biological sequences. J. Mol. Biol. 162: 705-708.
Henderson R, Baldwin JM, Ceska TA, Zemlin F, Beckmann E, Downing KH (1990). Model for the structure of bacteriorhodopsin based on high-resolution electron cryomicroscopy. J. Mol. Biol. 213: 899-929.
Henderson R, Schertler GF (1990). Philos Trans R Soc London, Ser B. 326: 379-389.
Heymann JAW, Subramaniam S (1997). Expression stability and membrane integration of truncation mutants of bovine rhodopsin. Proc. Natl. Acad. Sci. 94: 4966-4971.
Hooft RW, Vriend G, Sander C, Abola EE (1996a). Errors in protein structures. Nature. 381: 272. (http://swift.embl-hei-delberg. de/whatcheck/)
Lovell D IW, Arendall WB, Bakker PIW, de Word JM, Prisant MG, Richardson JS, Richardson DC (2003). Structure validation by Calpha geometry: phi, psi and Cbeta deviation. Proteins: Struct. Funct. Genet. 50: 437-450
Laskowski RA, MacArthur MW, Thornton JM (1998(). Validation of protein models derived from experiment. Curr. Opin. Struct. Biol. 8: 631-639
Li J, Edwards PC, Burghammer M, Villa C, Schertler GF (2004). Structure of bovine rhodopsin in a trigonal crystal form. J. Mol. Biol. 343: 1409-1438.
Nathans J, Hogness DS (1984). Isolation and Nucleotide Sequence of the Gene Encoding Human Rhodopsin. Proc. Natl. Academy of Sci. United States of Am. 81: 4851-4855.
Ogawa H, Toyoshima C (2002). Homology modeling of the cation binding sites of Na + K + ATPase. PNAS., 15977-15982
Okada T, Trong LI, Fox BA, Behnke CA, Stenkamp RE, Palczewski, K (2000). X-ray diffraction analysis of three-dimensional crystals of bovine rhodopsin obtained from mixed micelles. J. Struct Biol., 130: 73-80.
Okada T, Fujiyoshi Y, Silow M, Navarro J, Landau, EM, Shichida Y (2002). Functional role of internal water molecules in rhodopsin revealed by X-ray crystallography. Proc. Natl. Acad Sci USA. 99: 5982-5987.
Okada, T., Sugihara, M., Bondar, A.N., Elstner, M., Entel, P. and Buss, V., 2004. The retinal conformation and its environment in rhodopsin in light of a new 2.2 A crystal structure. J.Mol.Biol., 342: 571-583
Palczewski K (2000). Crystal structure of rhodopsin: a G protein-coupled receptor. Sci. 289: 733-734.
Pontius J, Richelle J, Wodak SJ (1996). Deviations from standard atomic volumes as a quality measure for protein crystal structures. J. Mol. Biol. 264: 121-136; (http://nihserver.mbi.ucla.edu/SAVS/)
Schertler GF (1993). Projection structure of rhodopsin. Nature. 362: 770-772.
Schertler GF, Hargrave PA (1995). Proc.Natl. Acad. Sci .USA. 92:11578-82.
Schertler GF (1999). Novartis Found Symp. 224: 54-66.
Schertler GF, Hargrave PA (2000). Preparation and analysis of two-dimensional crystals of Rhodopsin. Methods Enzymol. 315: 091-107.
Sali A, Blundell TL (1993). Comparative modelling by statisfaction of spatial restraints. J. Mol. Biol. 234: 779-815.
Teller DC, Okada T, Behnke CA, Palczewski K, Stenkamp RE (2001). Advances in determination of a high-resolution three-dimensional structure of rhodopsin, a model of G-protein-coupled receptors (GPCRs). Biochemistry. 40: 7761-7772.
Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673-4680.
Unger VM, Schertler GF (1995). Biophys. J. 68: 1776–86.
Unger VM, Hargrave PA, Baldwin JM, Schertler GF (1997). Arrangement of rhodopsin transmembrane α-helices. Nature. 389:203-206.
Vriend G (1998), Professtinal Gambling (http://swift.cmbi.kun.nl/gv/articles/text/gambling.html).
Xu L, Hazard ES, Lockman DK, Crouch RK, Ma J (1998). Molecular Cloning of the Salamander Red and Blue Cone Visual Pigments. Mol. Vision. 4:10.
Yeagle PL, Choi G, Albert AD (2001). Studies on the structure of the G-protein-coupled receptor rhodopsin including the putative G-protein binding site in unactivated and activated forms. Biochemistry. 40: 11932-11937.
Yokoyama S, Yokoyama R (1989). Molecular evolution of human visual pigment genes. Mol. Biol. Evol. 6: 186-197.