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Homology Modeling of Cysteinyl Leukotriene1 Receptor

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Abstract

Cysteinyl leukotrienes are inflammatory mediators having important role in pathophysiological conditions such as asthma, allergic rhinitis and have been implicated in a number of inflammatory conditions including cardiovascular and gastrointestinal diseases. Most of the disease regulatory actions of the CysLTs are mediated through CysLT1 receptor. Hence in the present study, homology modeling of CysLT1 was performed because the availability of 3D structure would enhance the development of new drugs for inflammatory diseases. However the templates identified have low sequence identity which increases the complexity of modeling. Hence, homology modeling was performed using single template, multiple templates and also using threading I-TASSER server. The best model was selected based on the validation of the generated models using Ramachandran and ERRAT plot. The model developed could be useful for identifying crucial residues and docking study.

Keywords: CysLTs, Homology Modeling.

1. Introduction

Cysteinyl-leukotrienes (CysLTs) are produced by the cells of immune system specially eosinophils, basophils, mast cells and macrophages[1, 2] and formed by the action of 5-lipoxygenase and its activating protein on arachidonic acid[3-9]. CysLTs are important mediators of inflammation and significantly modulate the inflammatory responses seen in asthma and allergic rhinitis[7-12]. The CysLTs are 7-transmembrane G-protein coupled receptors present on outer membrane of inflammatory cells having two subtypes namely CysLT1 and CysLT2[1-13] whose seven hydrophobic transmembrane domains are connected by six hydrophilic loops[10]. CysLTs are involved in maturation and migration of dendritic cells and selectively promote the generation of Th2 cytokines and hence enhances allergic responses[7,9].

Most of the disease regulatory actions of the CysLTs are mediated through CysLT1[7,8,11]. CysLT1 receptor has been localized both at gene and protein level in blood vessels and in the interstitial cells and highly expressed in spleen, peripheral blood leukocytes, interstitial lung macrophages and in airway smooth muscle[13]. It has been proved that CysLTR1 antagonists have a significant role in allergic rhinitis (AR) and asthma[1] and improves pulmonary and lung function, and provide a therapeutic alternative to glucocorticoids in patients with allergic airway disease[11]. The major interest for the development of antagonists of the CysLT1 receptor was because of their beneficial effects in the treatment of asthma, and zafirlukast, montelukast and pranlukast have been clinically introduced for this purpose[3, 6,11,13].

Homology modeling makes use of the fact that evolutionary related protein shares similar structures. Therefore the protein with unknown structure can be modeled using known structure (template) if both shares high sequence similarity. The three dimensional structure of CysLT1 is not reported to have been resolved. Hence, in this study we have generated models of cysteinyl leukotrienes1 receptor by three comparative modeling techniques namely, (i) simple homology modeling with single template, (ii) multi-template based homology modeling and (iii) threading with I-TASSER server. It was reported that the use of multiple templates in modeling of GPCR protein slightly increases the model quality[14] and therefore the multiple templates based modeling was performed.

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2. Material and Methods

2.1. Template Selection

The amino acid sequence of human cysteinyl leukotriene1 (accession No: Q9Y271) was retrieved from the Uniprot database. The three-dimensional structure of cysteinyl leukotriene1 is not yet available; hence the present study was undertaken. Protein BLAST[15] search was performed against PDB[16] with the default parameters to find suitable templates for homology modeling. Templates were selected based on sequence identity, query coverage and E-value. Multiple sequence alignment was done using CLUSTALW[17] program to find conserved residues.

2.2. Homology and Threading based Modeling of CysLT1

The three dimensional structures of CysLT1 were modeled using EasyModeller 4.0[18] which uses MODELLER 9.12[19] and Python 2.7.1 in the backend. Single

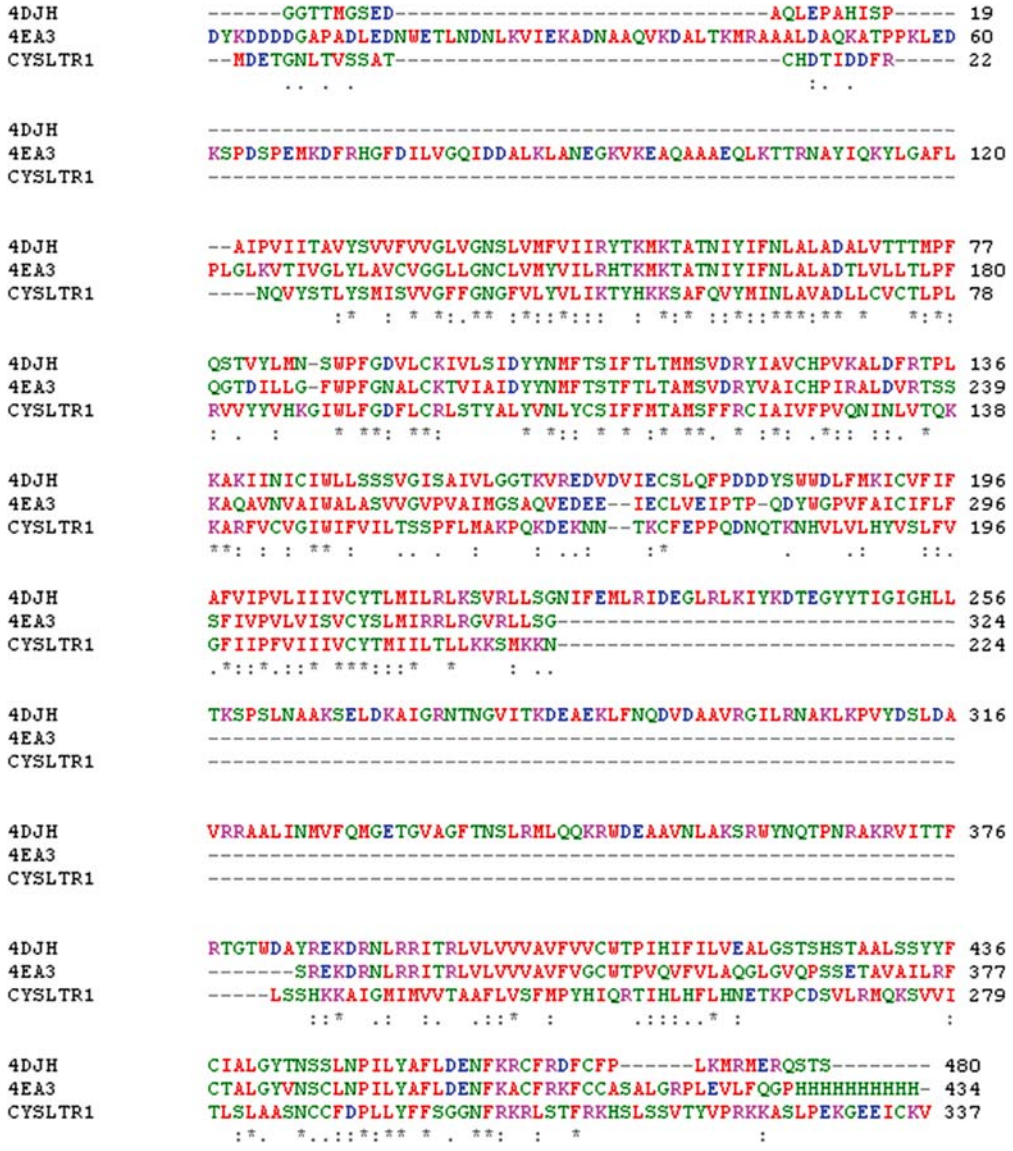

Fig. 1. Sequence alignment between target (CysLT1) and template (4DJH and 4EA3).

and multiple template based approaches were carried out using two different templates. The structure of cysteinyl leukotriene1 was also modeled used I-TASSER[20] server which is a protein structure modeling approach based on the secondary-structure enhanced profile-profile threading alignment (PPA) and the iterative implementation of the Threading ASSEmbly Refinement (TASSER) program. In this approach, the target sequence is first threaded through a PDB structure library to search for the possible folds by four simple variants of PPA methods employing the hidden Markov model, PSIBLAST profiles, Needleman-Wunsch and Smith-Waterman alignment algorithms.

2.3. Validation of CysLT1

The predicted models were validated using Ramachandran[21] and ERRAT plot[22]. The Ramachandran plot gives us information about the percentage of residues in allowed region. The ERRAT program is well suited for evaluating the progress of crystallographic model building and refinement and analyzes the statistics of non-bonded interactions between different atom types.

3. Results and Discussion

3.1. Template Selection

Sequence similarity is the major criteria to select the templates for homology modeling. The templates such as 4DJH, Crystal structure of human kappa opioid receptor with sequence identity 32%, query coverage 56% and 4EA3, Crystal structure of human nofq opioid receptor with sequence identity 29%, query coverage 86% were selected. Both templates belong to GPCR family having seven transmembrane helices topology. Since the query coverage and identity of the template was low, the multiple template based homology modeling was also performed to improve the model accuracy. The alignment between the target and templates were shown in Fig. 1.

Table 1. Validation of the generated model using RC plot and ERRAT plot

Model No	Template	Ramachandran Plot			ERRAT
		Favored (%)	Allowed (%)	Disallowed (%)	
1	4DJH	95.5	3.3	1.2	-
2	4DJH	94.6	3	2.4	-
3	4DJH	93.1	4.8	2.1	-
4	4DJH	94.9	2.4	2.7	-
5	4DJH	95.5	3.6	0.9	-
6	4DJH	95.8	3.0	1.2	-
7	4DJH	95.2	3.6	1.2	
8	**4DJH**	**96.7**	**2.4**	**0.9**	**71.66**
9	4DJH	95.5	2.7	1.8	-
10	4DJH, 4EA3	94.9	3.9	1.2	-
11	4DJH, 4EA3	94	3.9	2.1	-
12	4DJH, 4EA3	93.4	5.1	1.5	-
13	4DJH, 4EA3	92.8	5.7	1.5	-
14	4DJH, 4EA3	94.3	4.8	0.9	-
15	4DJH, 4EA3	93.1	5.4	1.5	-
16	4DJH, 4EA3	93.7	5.1	1.2	-
17	4DJH, 4EA3	93.7	4.5	1.8	-
18	**4DJH, 4EA3**	**96.4**	**3.0**	**0.6**	**62.73**
19	I-TASSER server	83	13.4	3.6	-
20	I-TASSER server	85.4	12.2	2.4	-
21	**I-TASSER server**	**89**	**9.3**	**1.8**	**92.56**
22	I-TASSER server	84.2	12.2	3.6	-
23	I-TASSER server	82.4	11.3	6.3	-

3.2. Model Generation

The three dimensional structure of CysLT1 is predicted using the comparative modeling program, Easy Modeller4.0 and online threading server I-TASSER. Using Easy Modeller, 9 models using Crystal structure of human kappa opioid receptor (4DJH) and 9 models using Crystal structure of human nofq opioid receptor (4EA3) and 4DJH were generated. The use of multiple templates generally increases model accuracy as it combines the information from multiple templates. Multiple template based approach was carried out with the aim of identifying the improvement in structure quality. The protein sequence of cysteinyl leukotriene1 was submitted to I-TASSER 3D structure prediction server, which produced five similar models. All the models were found to have 7-TM topology and the model with best c-score was chosen.

3.3. Model Validation

The predicted structures using different techniques were validated using Ramachandran (RC) and ERRAT plot. RC plot and ERRAT values were tabulated in Table 1. Based on validation results, one model is selected in each technique and is shown in Fig. 2 and

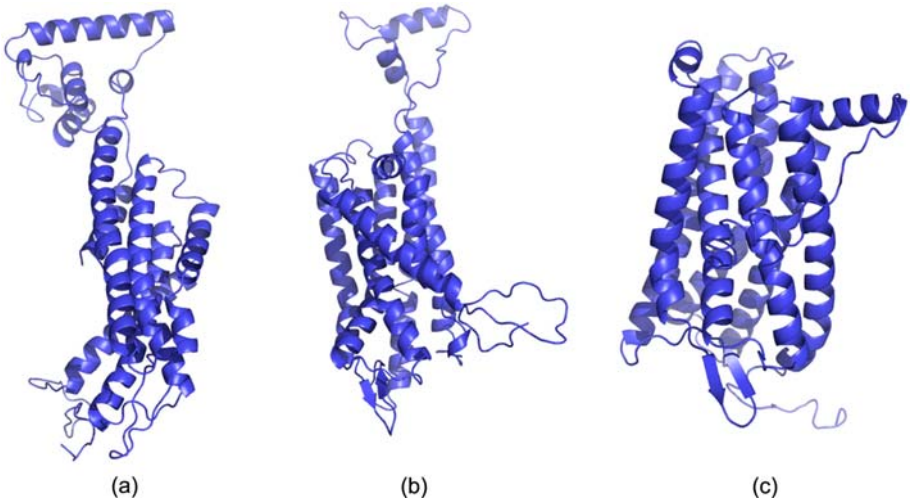

Fig. 2. Generated model for Cysteinyl leukotriene1. (a) Model generated using single template (4DJH) (b) Model generated using multiple template (4DJH, 4EA3) (c) Model generated using I-TASSER server.

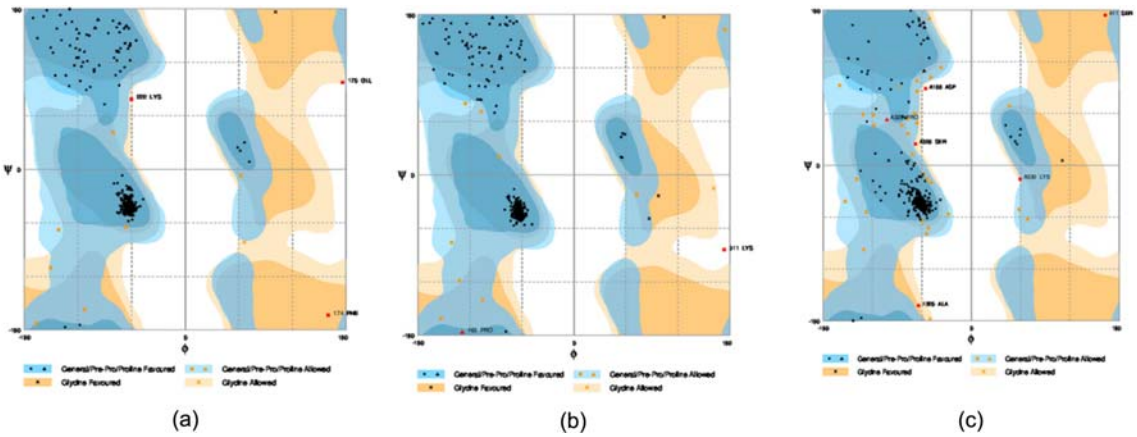

Fig. 3. Ramachandran plot for generated models. (a) Model generated using single template (4DJH) (b) Model generated using multiple template (4DJH, 4EA3) (c) Model generated using I-TASSER server.

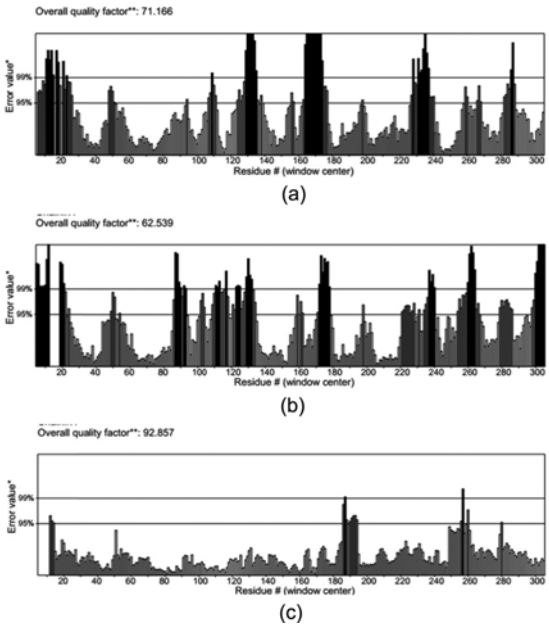

Fig. 4. Errat plot for the generated models. (a) Model generated using single template (4DJH) (b) Model generated using multiple template (4DJH, 4EA3) (c) Model generated using I-TASSER server.

its RC plot and ERRAT plot is illustrated in Fig. 3 and 4 respectively. The selected best model has 99.1%, 99.4% and 98.2% of residues in favored and allowed region and ERRAT showed overall quality factor of 71.6%, 62.7%, and 92.5% which validates the quality of the generated model from single template, multiple templates and I-TASSER respectively.

4. Conclusions

3D-models for cysteinyl leukotrienes1 receptor were generated using three different approaches. Our results demonstrate that homology modeling of cysteinyl leukotrienes1 with single and multiple templates shows somehow similar structure. The validation results suggest that all the generated models were reliable. The generated structures will serve as cornerstone for further analysis with cysteinyl leukotriene1 receptor.

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