3D Structure Prediction of Thromboxane A2 Receptor by Homology Modeling

Santhosh Kumar Nagarajan and Thirumurthy Madhavan†

Abstract

Thromboxane A2 receptors (TXA2-R) are the G protein coupled receptors localized on cell membranes and intracellular structures and play pathophysiological role in various thrombosis/hemostasis, modulation of the immune response, acute myocardial infarction, inflammatory lung disease, hypertension and nephrotic disease. TXA2 receptor antagonists have been evaluated as potential therapeutic agents for asthma, thrombosis and hypertension. The role of TXA2 in wide spectrum of diseases makes this as an important drug target. Hence in the present study, homology modeling of TXA2 receptor was performed using the crystal structure of squid rhodopsin and night blindness causing G90D rhodopsin. 20 models were generated using single and multiple templates based approaches and the best model was selected based on the validation result. We found that multiple template based approach have given better accuracy. The generated structures can be used in future for further binding site and docking analysis.

Keywords: TXA2 Receptor, Homology Modeling

1. Introduction

Thromboxane A2 receptor (TXA2-R) also known as the prostanoid TP receptor is one among the five classes of prostanoid receptors which belongs to G protein coupled receptor family[1-3]. TXA2 receptor is widely distributed among different organ systems and has been localized on both cell membranes and intracellular structures and exhibits a wide distribution in different cell types. TXA2 plays biological roles in diseases such as asthma, inflammatory lung disease, haemostasis/thrombosis, sickle cell disease, cardiovascular disease and lupus nephritis[2]. TXA2 receptor involvement in blood platelet function has received the greatest attention[1]. TXA2 receptor antagonists have been evaluated as potential therapeutic agents for asthma, thrombosis and hypertension[2,3]. The role of TXA2 in wide spectrum of diseases makes this as an important drug target.

Due to the substantial time required to prepare protein for crystallization, the number of protein structures resolved experimentally lags behind the sequence data available[4]. Homology Modeling provides a rapid way of predicting the three-dimensional structure of a protein using sequence data when no crystal structure is available. Thromboxane A2 receptor does not have crystal structure therefore in this study the 3D structure of TXA2-R was predicted by single and multiple template based homology modeling[5]. 20 models were generated and validated using Ramachandran plot and ERRAT values. The predicted model would be useful for the development of new drugs for the TXA2-R based diseases.

2. Material and Methods

2.1. Template Selection

The amino acid sequence of human Thromboxane A2 receptor (accession No:P21731) was retrieved from the Uniprot database. A theoretical model of the Thromboxane A2 receptor is available in the PDBe database. But, PDB no longer accepts theoretical model, hence this study was initiated. Protein BLAST[6] search was performed against PDB[7] to find suitable templates for homology modelling. Templates were selected based on sequence identity, query coverage and E-value. It has
been statistically proven that if the level of sequence identity is above 30%, then up to 90% of the polypeptide conformation tends to be modelled well [4]. Below 30% identity models are unreliable due to increasing alignment errors [4]. Multiple sequence alignment was done using CLUSTALW [8] program to find conserved residues.

### 2.2. Modelling and Validation of TXA2 Receptor

The three dimensional structures of TXA2-R were modelled using EasyModeller 4.0 [9] which uses MOD-ELLER 9.12 [9] and Python 2.7.1 in the backend. Single and multiple template based approaches were carried out using three different templates. The predicted models were assessed and validated using the RMSD values.

**Table 1.** The query coverage and identity values of the templates

| PDB ID | Max Score | Total score | Query Coverage % | E Value | Identity % |
|--------|-----------|-------------|-----------------|---------|------------|
| 2ZIY   | 49.7      | 49.7        | 32 %            | 2e-06   | 28%        |
| 2Z73   | 49.3      | 49.3        | 32 %            | 2e-06   | 28%        |
| 4BEZ   | 41.2      | 81%         | 32 %            | 9e-04   | 22%        |

**Fig. 1.** Alignment between the target (TBXA2R) and templates (2ZIY, 2Z73, 4BEZ).
Later, the validation by programmes such as Ramachandran plot and ERRAT was carried out. Using RAMPAGE server, Ramachandran plot is plotted for each of the predicted models which depicts the percentage of amino acids in favoured region. ERRAT is a protein structure verification algorithm that is especially well-suited for evaluating the progress of crystallographic model building and refinement.

3. Results and Discussion

3.1. Template Selection
The computational method of building the model involves the template selection, alignment of the target with the template, model building and validation. The templates such as crystal structure of squid rhodopsin (2ZIY and 2Z73), crystal structure of night blindness causing G90D rhodopsin (4BEZ) were selected for modeling. The top templates obtained from blast search are shown in Table 1. The multiple template based homology modelling was also performed to check whether this approach could improve the model accuracy in this case. The alignment between the target and template is shown in Fig. 1.

3.2. Model Generation and Validation
Model was generated using single and multiple template based approaches. Easy Modeller 4.0 was used for model generation in this study. Using the templates 2ZIY, 2Z73 and 4BEZ, 5 models were generated for each template separately. Multiple template based approach was performed using all three templates and 5 models were generated. Hence, a total of 20 models were generated in this study. Initially the RMSD between the generated models and the templates were calculated. ERRAT and Ramachandran plot analysis was performed to evaluate the quality of the generated models. All the validation results were tabulated in Table 2. Based on criteria such as low RMSD, high percentage of residues in favoured and allowed regions and high ERRAT quality value the best model was chosen within each template based model. No model was

| Model No | Template | RMSD | Ramachandran Plot | ERRAT quality value |
|----------|----------|------|-------------------|---------------------|
|          |          |      | Favoured (%)      | Allowed (%)         | Disallowed (%)      |
| 1        | 2ZIY     | 0.294| 93.0              | 5.6                 | 1.5                 | 69.725               |
| 2        | 2ZIY     | 0.398| 92.7              | 6.2                 | 1.2                 | 70.571               |
| 3        | 2ZIY     | 0.306| 95.0              | 3.8                 | 1.2                 | 70.909               |
| 4        | 2ZIY     | 0.335| 94.4              | 3.8                 | 1.8                 | 66.061               |
| 5        | 2ZIY     | 0.326| 93.0              | 5.0                 | 2.1                 | 62.575               |
| 6        | 2Z73     | 1.424| 97.1              | 1.8                 | 1.2                 | 77.313               |
| 7        | 2Z73     | 1.423| 94.7              | 4.7                 | 2.0                 | 75.821               |
| 8        | 2Z73     | 1.516| 93.8              | 5.9                 | 0.3                 | 80.000               |
| 9        | 2Z73     | 1.420| 95.6              | 2.9                 | 1.5                 | 77.313               |
| 10       | 2Z73     | 1.770| 95.3              | 3.5                 | 1.2                 | 77.015               |
| 11       | 4BEZ     | 4.776| 92.4              | 5.6                 | 2.1                 | 64.865               |
| 12       | 4BEZ     | 4.853| 95                 | 4.4                 | 0.6                 | 62.575               |
| 13       | 4BEZ     | 4.702| 92.7              | 5.6                 | 1.8                 | 59.639               |
| 14       | 4BEZ     | 4.704| 92.7              | 6.2                 | 1.2                 | 65.075               |
| 15       | 4BEZ     | 5.036| 95.6              | 3.5                 | 0.9                 | 70.871               |
| 16       | 2ZIY, 2Z73, 4BEZ | 0.423| 95.3              | 3.2                 | 1.5                 | 82.934               |
| 17       | 2ZIY, 2Z73, 4BEZ | 0.645| 94.4              | 4.1                 | 1.5                 | 77.313               |
| 18       | 2ZIY, 2Z73, 4BEZ | 0.403| 96.2              | 3.2                 | 0.6                 | 77.313               |
| 19       | 2ZIY, 2Z73, 4BEZ | 0.398| 95.3              | 3.2                 | 1.5                 | 75.522               |
| 20       | 2ZIY, 2Z73, 4BEZ | 0.350| 95                 | 3.2                 | 1.8                 | 79.403               |
selected using 4BEZ as template, as it contained large RMSD and low ERRAT quality. 3 models were selected as final model and they are represented in Fig. 2 and their RC plot is shown in Fig. 3 and ERRAT plot in Fig. 4. The RMSD between these selected models was found to be 0.782. The generated model has retained the 7 helices which is the characteristic feature of GPCR family. Of the three selected models we found that the model obtained using multiple template found to be better in validation which shows that multiple template based approach has enhanced quality.

4. Conclusion

Three dimensional models for Thromboxane A2 receptor (TXA2-R) were generated using single and multiple template based approaches. Models generated using 2ZIY and 2Z73 are found to be reliable. Homology modeling with multiple templates shows enhanced accuracy. Further, these models could be used for binding site analysis and docking and has potential advantage in rational drug design for TXA2-R implicated diseases.
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