Homology Modeling of Chemokine Receptor CXCR3: A Novel Therapeutic Target against Inflammatory Diseases

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Abstract

CXCR3 is a C-X-C chemokine receptor type 3 also known as GPR9 and CD183. CXCR3 is a G-Protein coupled chemokine receptor which interacts with three endogenous interferon inducible chemokine’s (CXCL9, CXCL10 and CXCL11) and is proved to play a vital role in the Th1 inflammatory responses. CXCR3 has been implicated to be associated with various disease conditions like inflammatory diseases, autoimmune diseases, Type I diabetes and acute cardiac allograft rejection. Therefore CXCR3 receptor is found to be an attractive therapeutic target for the treatment of inflammatory diseases. In order to decipher the biological function of a CXCR3, 3D structure is of much importance but the crystal structure for CXCR3 has not yet been resolved. Hence, in the current study Homology modeling of CXCR3 was performed against various templates and validated using different parameters to suggest the best model for CXCR3. The reported best model can be used for further studies such as docking to identify the important binding site residues.

Keywords: CXCR3, Homology Modeling

1. Introduction

CXCR3 is a chemokine receptor belonging to the superfamily of seven transmembrane spanning G-protein coupled receptors (GPCRs) [1,2]. CXCR3 is found to be predominantly expressed on activated T-cells of type 1 (Th1) and it helps in activation and recruitment of Th1 cells[3-5]. In the recent studies it’s been reported that CXCR3 is also found to be expressed on NK cells, B cells and partially on circulating CD4+ and CD8+ T cells[6]. CXCR3 binds with its specific natural ligands (CXCL9, CXCL10 and CXCL11) and plays an important role of directing the Th1 cells to the site of inflammation/immune injury[7,8]. These ligands are produced by the activated leukocytes (monocytes and macrophages), vascular endothelial cells and activated tissue cells[9]. CXCR3 and its ligands are found to be highly expressed over a broad spectrum of diseases conditions like inflamed joints of rheumatoid arthritis patients[10], multiple sclerosis lesion in the brain[11], asthma[12], psoriasis[13], tumor metastasis[14], Type 1 diabetes[14] and in cardiac allograft rejection[15].

Thereby CXCR3 receptor’s involvement over a wide range of diseases has made it to be an attractive therapeutic target of interest for the treatment of the above diseases. The three dimensional structure of CXCR3 is an essential component in the perspective of understanding its underlying biological functions at molecular level. In the current studies Homology model for CXCR3 receptor is built against multiple templates and 27 models were successfully generated, as there is no resolved crystal structure in PDB. These 27 models were validated using different parameters such as RMSD, ERRAT and Ramachandran plot scores to identify and suggest the best model for performing the further docking studies for the identification of the important binding site residues.

2. Experimental Section

2.1. Template Selection and Sequence Analysis for CXCR3

Template selection is the most critical start point in homology modeling because the selected template directly determines the main folding of the target structures and influences the quality of the generated homologous model. In the current model template selection is performed against multiple templates and 27 models were successfully generated. These models were validated using different parameters such as RMSD, ERRAT and Ramachandran plot scores to identify and suggest the best model for further studies.

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ology model. Due to several limitations and difficulties in resolving the crystal structure for the membrane-bound proteins, there are only few experimentally (via X-ray crystallography or NRM) well resolved three dimensional structures are available in PDB. Therefore in our current studies we obtain the amino acid sequence for the human CXCR3 from the Uniprot database (P49682). The retrieved sequence of human CXCR3 was submitted to NCBI blast\(^{[16]}\) tool to perform a search against the PDB\(^{[17]}\) database. Three best templates were chosen based upon the major template selection criteria’s like high percentage of sequence identity, maximum query coverage, lower – E value and template crystal structure resolution. The sequence alignment between the chosen templates and the target is the next crucial step in the homology modeling as in the accuracy of the alignment directly influences the accuracy of the quality of the generated homology models. In this approach multiple sequence alignment is suggested to be the best and sequence alignment was performed using ClustalW2.1 tool\(^{[18]}\). The gap regions in the alignment usually indicates residue variations between the template and target sequences, thereby regions showing less gaps are more conserved and preferred regions.

2.2. Homology Modeling and Validation

The ultimate goal of protein modeling is to predict the structure from its sequence with an accuracy that is comparable to the best results achieved experimentally. This would allow users to safely use a rapidly generated in-silico protein models to obtain the structural information if the experimental technique fails. In the current study the homology model for human CXCR3 protein was successfully built using EASY MODELLER 4.0\(^{[19]}\) which is GUI based tool for homology modeling which uses MODELLER 9.12\(^{[20]}\) and Python 2.7.1 in the back-end. The quality of the generated models were validated based on various scores such as RMSD value calculated by superimposing the target and template structure\(^{[21]}\), Ramachandran plot generated using RAMPAGE tool\(^{[22]}\) and ERRAT\(^{[23]}\). The Ramachandran plot checks for the stereo-chemical quality of a protein by analyzing residue-by- residue geometry and overall structure geometry to indicate how well the structure has been determined. The ERRAT program works by analysing the statistics of non-bonded interactions between different atom types, with higher scores indicating higher quality. The RMSD value (Root mean square deviation) is the measure of the average distance between the atoms, usually the backbone atoms of superimposed proteins. Regions with high RMSD values are those that are not correctly represented by the data.

3. Results and Discussion

3.1. Sequence Analysis of CXCR3

BLAST search was performed effectively against the protein data bank (PDB) for selecting an appropriate template for the target protein CXCR3. The blast search fetched out broad list of templates for CXCR3, among which the three templates that satisfying the template selection criteria’s such as sequence identity >30%, maximum query coverage and template crystal structure with good resolution of < 4 angstrom. [Table 1]. All the three templates chosen from the blast results belongs to (3ODU, 3OE6 & 3OE0) human CXCR4 resolved protein structure. In this case, the crystal structure of CXCR4 does not contain the N-terminal domain, and hence, we have modeled the N-terminal part of CXCR3 using the Bovine rhodopsin template (1F88_A) combine along with the template structure of CXCR3 (3ODU_A) this method is based upon the using multiple templates to generate a models. The other two templates of human CXCR4 (3OE6_A & 3OE0_A) weren’t combined along with the rhodopsin template for generating a models. The alignment of target CXCR3 with different templates was shown in Fig. 1-3.

3.2. Homology Modeling and Validation

The three dimensional structure of CXCR3 target protein was modeled using EASY MODELLER 4.0. A total of 27 models were generated (9 models per template).
Fig. 1. Sequence alignment between the CXCR3 (target) and with two templates CXCR4 (3ODU_A) and rhodopsin (1F88_A).

Fig. 2. Sequence alignment between the CXCR3 (target) and the template CXCR4 (3OE6_A).
Fig. 3. Sequence alignment between the CXCR3 (target) and the template CXCR4 (3OE0_A).

Table 2a. The results of the generated models using the multiple templates (3ODU_A & 1F88_A) were validated against RMSD, Ramachandran plot and ERRAT values

| Model No | RMSD Value | Ramachandran Plot | Errat value |
|----------|------------|--------------------|-------------|
|          |            | Favored | Allowed | Disallowed |             |
| 1.       | 0.466      | 91.0 %  | 5.2 %   | 3.8 %      | 72.145      |
| 2.       | 0.422      | 92.9 %  | 4.6 %   | 2.5 %      | 63.944      |
| 3.       | 0.413      | 92.1 %  | 5.2 %   | 2.7 %      | 69.444      |
| 4.       | 0.425      | 92.6 %  | 4.1 %   | 3.3 %      | 67.877      |
| 5.       | 0.435      | 92.9 %  | 4.9 %   | 2.2 %      | 63.585      |
| 6.       | 0.388      | 92.6 %  | 5.5 %   | 1.9 %      | 68.627      |
| 7.       | 0.448      | 92.9 %  | 5.2 %   | 1.9 %      | 62.778      |
| 8.       | 0.375      | 92.9 %  | 4.6 %   | 2.5 %      | 66.854      |
| 9.       | 0.442      | 92.9 %  | 4.1 %   | 3.0 %      | 66.006      |

Table 2b. The results of the generated models using the template (3OE6_A) were validated against RMSD, Ramachandran plot and ERRAT values

| Model No | RMSD Value | Ramachandran Plot | ERRAT value |
|----------|------------|--------------------|-------------|
|          |            | Favored | Allowed | Disallowed |             |
| 10.      | 2.628      | 93.7 %  | 4.9 %   | 1.4 %      | 64.327      |
| 11.      | 2.198      | 94.3 %  | 3.6 %   | 2.2 %      | 64.497      |
| 12.      | 1.805      | 93.7 %  | 4.1 %   | 2.2 %      | 66.154      |
| 13.      | 1.967      | 94.5 %  | 4.1 %   | 1.4 %      | 71.429      |
| 14.      | 1.947      | 92.3 %  | 5.2 %   | 2.5 %      | 59.317      |
| 15.      | 1.695      | 93.4 %  | 4.6 %   | 1.9 %      | 64.263      |
| 16.      | 2.018      | 94.0 %  | 4.4 %   | 1.6 %      | 70.122      |
| 17.      | 2.427      | 95.1 %  | 3.3 %   | 1.6 %      | 66.769      |
| 18.      | 2.192      | 92.3 %  | 6.8 %   | 0.8 %      | 64.035      |
Table 2c. The results of the generated models using the template (3OE0_A) were validated against RMSD, Ramachandran plot and ERRAT values

| Model No | RMSD Value | Ramachandran Plot | Errat value |
|----------|------------|--------------------|-------------|
|          |            | Favored | Allowed | Disallowed |             |
| 19.      | 1.283      | 92.9 %  | 5.2 %   | 1.9 %      | 73.731      |
| 20.      | 1.130      | 92.6 %  | 6.0 %   | 1.4 %      | 65.057      |
| 21.      | 1.221      | 92.3 %  | 5.5 %   | 2.2 %      | 63.352      |
| 22.      | 1.080      | 94.3 %  | 3.6 %   | 2.2 %      | 75.504      |
| 23.      | 0.973      | 89.6 %  | 6.6 %   | 3.8 %      | 75.075      |
| 24.      | 1.062      | 93.4 %  | 4.1 %   | 2.5 %      | 67.372      |
| 25.      | 1.222      | 91.3 %  | 6.6 %   | 2.2 %      | 70.339      |
| 26.      | 1.353      | 94.0 %  | 3.8 %   | 2.2 %      | 65.266      |
| 27.      | 1.205      | 92.6 %  | 5.7 %   | 1.6 %      | 64.689      |

Fig 4a. The generated models were superimposed with template 3ODU & 1F88 and RMSD value was calculated. The green color model indicates the generated model and cyan color indicates the template 3ODU & 1F88.
It was found that the seven transmembrane have been properly transformed according to the template structure only for the models generated using the multiple templates of human CXCR4 (3ODU_A) and Bovine rhodopsin (1F88). The RMSD value was calculated for all the 27 models by superimposing the template and target structure and the RMSD value for a good model should be less than \(< 4\) angstrom. The further validation for all the 27 models were performed using RAMPAGE tool to calculate the stereo-chemical properties of the model and ERRAT program was performed for all the generated models to analyze the overall quality factor of the models. Higher the ERRAT value, better the quality of the model. All the validation results are all tabulated on Table 2 (a, b & c).

The superimposed structures of the template and target model for calculating the RMSD values, the Ramachandran plot and the ERRAT
program predicting the overall quality of the structures are illustrated on Fig. 4(a, b & c), Fig. 5(a, b & c) and Fig. 6(a, b & c). The best model chosen out of the 27 generated homology model was **Model No: 1**, has it was found to satisfy all the validation criteria’s like having 91% of residues in the favored region, overall quality of the model was 72% and having a RMSD score of 0.466. The above validation scores obtained by the chosen model proved it to be an appropriate three dimensional model for CXCR3 target protein.

**Fig. 4c.** The generated models were superimposed with template 3OE0 and RMSD value was calculated. The green color model indicates the generated model and cyan color indicates the template 3OE0.

### 4. Conclusion

This study has presented a homology model of human CXCR3 for better understanding of the biological function of the protein. Models were constructed using three different templates in order to acquire a reliable model for CXCR3. The best model was chosen based on validation. We hope that our model could be useful for identifying critical residues and for designing new inhibitors.
Fig. 5a. Stereo-chemical quality validation of the generated models using Ramachandran plot.

Fig. 5b. Stereo-chemical quality validation of the generated models using Ramachandran plot.
Fig. 5c. Stereo-chemical quality validation of the generated models Ramachandran plot.

Fig. 6a. Validating the overall quality of the generated models using ERRAT program.
Fig. 6b. Validating the overall quality of the generated models using ERRAT program.

Fig. 6c. Validating the overall quality of the generated models using ERRAT program.
References

[1] Y. Wanga, J. Busch-Petersen, F. Wang, T. J. Kiesow, T. L. Graybill, J. Jin, Z. Yang, J. J. Foley, G. E. Hunsberger, D. B. Schmidt, H. M. Sarau, E. A. Capper-Spudich, Z. Wu, L. S. Fisher, M. S. McQueney, R. A. Rivero, and K. L. Widdowson, Camphor sulfonamide derivatives as novel, potent and selective CXCR3 antagonists, Bioorg. Med. Chem. Lett., Vol. 19, pp. 114-118, 2009.

[2] A. G. Nair, M. K. C. Wong, Y. Shu, Y. Jiang, C.-H. Jenh, S. H. Kim, D.-Y. Yang, Q. Zeng, Y. Shao, L. G. Zawacki, J. Duo, B. F. McGuinness, C. D. Carroll, D. W. Hobbs, N.-Y. Shih, S. B. Rosenblum, and J. A. Kozlowski, “Discovery of CXCR3 antagonists substituted with heterocycles as amide surrogates as novel, potent and selective CXCR3 antagonists”, Bioorg. Med. Chem. Lett., Vol. 19, pp. 114-118, 2009.

[3] J. Liu, Z. Fu, A.-R. Li, M. Johnson, L. Zhu, A. Marcus, J. Danao, T. Sullivan, G. Tonn, T. Collins, and J. Medina, Optimization of a series of quinazoline-derived antagonists of CXCR3, Bioorg. Med. Chem. Lett., Vol. 19, pp. 5114-5118, 2009.

[4] Y. Shao, G. N. Anilkumar, C. D. Carroll, G. Dong, J. W. Hall III, D. W. Hobbs, Y. Jiang, C.-H. Jenh, S. H. Kim, J. A. Kozlowski, B. F. McGuinness, S. B. Rosenblum, I. Schulman, N.-Y. Shih, Y. Shao, M. K. C. Wong, W. Yu, L. G. Zawacki, and Q. Zeng, SAR studies of pyridyl-piperazinyl-piperidine derivatives as CXCR3 chemokine antagonists, Bioorg. Med. Chem. Lett., Vol. 21, pp. 1527-1531, 2011.

[5] A. G. Cole, I. L. Stroke, M.-R. Brescia, S. Simhadri, J. J. Zhang, Z. Hussain, M. Snider, C. Haskell, S. Ribeiro, K. C. Appell, I. Henderson, and M. L. Webb, Identification and initial evaluation of 4-N-aryl-[1, 4] diazepane ureas as potent CXCR3 antagonists, Bioorg. Med. Chem. Lett., Vol. 16, pp. 200-203, 2006.

[6] X. Chen, J. Mihalic, J. Deignan, D. J. Gustin, J. Duquette, X. Du, J. Chan, Z. Fu, M. Johnson, A.-R. Li, K. Henne, T. Sullivan, B. Lemon, J. Ma, S. Miao, G. Tonn, T. Collins, and J. C. Medina, Discovery of potent and specific CXCR3 antagonists, Bioorg. Med. Chem. Lett., Vol. 22, pp. 357-362, 2012.

[7] G. Thoma, R. Baenteli, I. Lewis, T. Wagner, L. Oberer, W. Blum, F. Glickman, M. B. Streiff, and H.-G. Zerwes, Special ergolines are highly selective, potent antagonists of the chemokine receptor CXCR3: Discovery, characterization and preliminary SAR of a promising lead, Bioorg. Med. Chem. Lett., Vol. 19, pp. 6185-6188, 2009.

[8] X. Du, X. Chen, J. T. Mihalic, J. Deignan, J. Duquette, A.-R. Li, B. Lemon, J. Ma, S. Miao, K. Ebsworth, T. J. Sullivan, G. Tonn, T. L. Collins, and J. C. Medina, “Design and optimization of imidazole derivatives as potent CXCR3 antagonists”, Bioorg. Med. Chem. Lett., Vol. 18, pp. 608-613, 2008.

[9] G. Thoma, R. Baenteli, I. Lewis, D. Jones, J. Kova-rick, M. B. Streiff, and H.-G. Zerwes, Special ergolines efficiently inhibit the chemokine receptor CXCR3 in blood, Bioorg. Med. Chem. Lett., Vol. 21, pp. 4745-4749, 2011.

[10] S. Storelli, P. Verdijk, D. Verzijl, H. Timmerman, A. C. van de Stolpe, C. P. Tensen, M. J. Smit, I. J. P. De Esch, and R. Leurs, Synthesis and structure–activity relationship of 3-phenyl-3H-quinazolin-4-one derivatives as CXCR3 chemokine receptor antagonists, Bioorg. Med. Chem. Lett., Vol. 15, pp. 2910-2913, 2005.

[11] G. Thoma, R. Baenteli, I. Lewis, T. Wagner, L. Oberer, W. Blum, F. Glickman, M. B. Streiff, and
H.-G. Zerwes, Special ergolines are highly selective, potent antagonists of the chemokine receptor CXCR3: Discovery, characterization and preliminary SAR of a promising lead, Bioorg. Med. Chem. Lett., Vol. 19, pp. 6185-6188, 2009.

[12] B. Homey, Chemokines and chemokine receptors as targets in the therapy of psoriasis, Curr. Drug Targets-Inflammation & Allergy, Vol. 3, pp. 169-174, 2004.

[13] X. Ma, K. Norsworthy, N. Kundu, W. H. Rodgers, P. A. Gimotty, O. Goloubeva, M. Lipsky, Y. Li, D. Holt, and A. Fulton, CXCR3 expression is associated with poor survival in breast cancer and promotes metastasis in a murine model, Mol. Cancer Ther., Vol. 8, pp. 490-498, 2009.

[14] S. H. Kim, G. N. Anilkumar, L. G. Zawacki, Q. Zenga, D.-Y. Yang, Y. Shao, G. Dong, X. Xu, W. Yu, Y. Jiang, C.-H. Jenh, J. W. Hall III, C. D. Carroll, D. W. Hobbs, J. J. Baldwin, B. F. McGuinness, S. B. Rosenblum, J. A. Kozlowski, B. B. Shankar, N.-Y. Shih, III. Identification of novel CXCR3 chemokine receptor antagonists with a pyrazinyl-piperazinyl-piperidine scaffold, Bioorg. Med. Chem. Lett., Vol. 21, pp. 6982-6986, 2011.

[15] S. Bastani, W. Sherman, G. T. Schnickel, G. R. Hsieh, George, R. Bhatia, M. C. Fishbein, A. Ardehal, “Chemokine receptor blockade with a synthetic non-peptide compound attenuates cardiac allograft vasculopathy, Transplantation, Vol. 88, pp. 995-1001, 2009.

[16] S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, “Basic local alignment search tool, J. Mol. Biol., Vol. 215, pp. 403-410, 1990.

[17] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, and P. E. Bourne, “The protein data bank, Nucleic Acids Res., Vol. 28, pp. 235-242, 2000.

[18] J. D. Thompson, D. G. Higgins, and T. J. Gibson, “CLUSTAL W: improving the sensitivity of progressive sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res., Vol. 22, pp. 4673-4680, 1994.

[19] B. K. Kuntal, P. Aparoy, and P. Reddanna, “Easy modeller: A graphical interface to modeller, BMC Research Notes, Vol. 3, pp. 226, 2010.

[20] N. Eswar, M. A. Marti-Renom, B. Webb, M. S. Madhusudhan, D. Eramian, M. Shen, U. Pieper, and A. Sali, “Comparative protein structure modelling with Modeller, Current Protocols in Bioinformatics, Vol. 5, pp. 1-5, 2006.

[21] A. Bagaria, V. Jaravine, Y. J. Huang, G. T. Montelione, and P. Güntert, “Protein structure validation by generalized linear model root-mean-square deviation prediction, Protein Sci., Vol. 21, pp. 229-238, 2012.

[22] S. A. Hollingsworth and P. A. Karplus, A fresh look at the Ramachandran plot and the occurrence of standard structures in proteins, Biomolecular Concepts, Vol. 1, pp. 3-4, 2010.

[23] C. Colovos and T. O. Yeates, “Verification of protein structures: patterns of nonbonded atomic interactions, Protein Sci., Vol. 2, pp. 1511-1519, 1993.