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Homology Modeling of the Calcium Sensing Receptor Extracellular Domain

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Summary

The extracellular calcium-sensing receptor (CaR) on the parathyroid cell surface negatively regulates secretion of parathyroid hormone (PTH). CaR has an important rule in diseases. Cinacalcet hydrochloride, an allosteric agonist of this receptor was approved by FDA in 2004 for treatment of secondary hyperparathyroidism. Cinacalcet is indicated for the treatment of hypercalcemia in patients with parathyroid carcinoma or for secondary hyperparathyroidism in patients with chronic kidney disease who require dialysis. This drug is an allosteric agonist which is bind to transmembrane domain of CaR. Studies showed that the ligand binding region of CaR, as expected, is located at the amino-terminal domain (extracellular domain). There is no crystal structure or model is available for this domain of CaR so we constructed the model and found putative ligand binding site. This model will be useful for finding new CaR orthosteric ligands and designing new drugs.

Key Words: calcium sensing receptor, PTH, homology modeling, cinacalcet, hyperparathyroidism, hypercalcemia, parathyroid carcinoma, kidney disease.

Introduction

Hormones such as parathormone (PTH), 1,25-dihydroxyvitamin D3 and calcitonin, interacting with their respective target organs and tissues involved in the regulation of calcium homeostasis, were promptly recognized as major participants in the adequate maintenance of this delicate balance. However, the presumed common mechanism responsible for the sensing of minor variations in extracellular calcium concentration could be only successfully identified with the studying of the CaR(1).

The human homologue of the CaR is a G protein-coupled receptor (GPCR) consisting of 1078 amino acid residues. The first 612 amino acids are included in a large extracellular domain (ECD), which is a feature of the subfamily to which the CaR belongs(also called family C or family 3 (1).

Since the CaR represents a potential therapeutic target for disorders in which the receptor is inappropriately overactive or underactive, some compounds have been developed with the aim of either activating (calcimimetics) or inactivating (calcilytics) ameliorated by the administration of calcimimetics (2).

Cinacalcet is a type II calcimimetic agent with a novel mechanism of action. It binds to the transmembrane region of the calcium-sensing receptor, (allosteric agonist) which leads to a different structural configuration that is more sensitive to serum calcium. Unlike vitamin D sterols, cinacalcet does not increase serum calcium levels; therefore, adverse effects associated with hypercalcemia can be avoided (3).Cinacalcet corrected hypercalcemia and improved phosphatemia in patients with persistent hyperparathyroidism after transplantation with no negative effects on renal function (4).

Due to finding new drugs in this class, transmembrane region of CaR is modeled in 2004(5) and refined in 2007 (6).

The agonist-binding domain of the calcium-sensing receptor is located at the amino-terminal domain(7). In this study we constructed the 3D model of extracellular domain of CaR by homology modeling method.

Comparative modeling methods, when applicable, provide the most reliable and accurate protein structure models .Comparative modeling is based on the general observation that evolutionarily related sequences have similar three-dimensional structures. As a consequence, a three-dimensional model of a protein of interest (target) can be built from related protein(s) of known structure [template(s)] that share statistically significant sequence similarity (at least 30%) (7).

Methods

First we retrieved the target sequence. The amino acid sequence of the extracellular calcium sensing receptor was obtained from the EXPASY server http://au.expasy.org/uniprot/P41180) (primary accession number: P41180). It was ascertained that the three-dimensional structure of the protein was not available in Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), hence the present exercise of developing the 3D model of the extracellular domain of CaR was undertaken. The protein has 1078 amino acids with a molecular weight of ~120 kDa. Then we got residues 1 to 612 (extracellular domain) (8). we searched similar sequences, using Basic Local Alignment and Search Tool in NCBI against Protein Data Bank (PDB) (http://www.ncbi.nlm.nih.gov/BLAST/) (9). From the homology searching, three templates were selected. Amino acid sequence alignment of target and template proteins was derived using the ClustalW program (http://www.ebi.ac.uk/Tools/clustalw/). Default parameters were applied and the aligned sequences were edited by JALVIEW program .A rough 3-D model was constructed from the sequence alignment between CaR and the template proteins using MODELLER 9v2 (http://salilab.org/modeller/) with parameters of energy minimization value. This program obtains a 3D model by optimization of a molecular probability density function (pdf). The molecular pdf for comparative modeling is optimized with the variable target function procedure in Cartesian space that employs methods of conjugate gradients and molecular dynamics with simulated annealing (10). The three loops in models which were found in model (using DOPE potential profile) were refined by loop-model module of MODELLER program

In the last step of homology modeling the refined structure of the model was subjected to a series of tests for testing its internal consistency and reliability. Backbone conformation was evaluated by the inspection of the Psi/Phi Ramachandran

plot obtained from PROCHECK analysis program in SWISS MODEL WORKSPACE (http://swissmodel.expasy.org/workspace/) The PROCHECK suite of programs assess the "stereochemical quality" of a given protein structure. The aim of PROCHECK is to assess how normal, or conversely how unusual, the geometry of the residues in a given protein structure is, as compared with stereochemical parameters derived from well-refined, high-resolution structures(11). Three-dimensional profiles of protein structure was investigated by the calculation of VERIFY-3D program in SWISS MODEL WORKSPACE (http://swissmodel.expasy.org/workspace/) The VERIFY-3D method assess protein structures using three-dimensional profiles. This program analyzes the compatibility of an atomic model (3D) with its own amino acid sequence (1D). Each residue is assigned a structural class based on its location and environment (alpha, beta, loop, polar, non polar etc). Then a database generated from good structures is used to obtain a score for each of the 20 amino acids in this structural class. The vertical axis in the plot represents the average 3D-1D profile score for each residue in a 21-residue sliding window. The scores ranges from -1 (bad score) to +1 (good score) (12). Finally we used three programs for finding putative binding sites in this model: CASTp server which is using the weighted Delaunay triangulation and the alpha complex for shape measurements. It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules. (http://sts.bioengr.uic.edu/castp/index.php) (13); Pocket-Finder which is a pocket detection algorithm based on Ligsite. Pocket-Finder works by scanning a probe radius 1.6 angstoms along all gridlines of a grid resolution 0.9 angstroms surrounding the protein. The probe also scans cubic diagonals. (http://bioinformatics.leeds.ac.uk/qsitefinder/) (14); Q-SiteFinder which is a new method of ligand binding site prediction. It works by binding hydrophobic (CH3) probes to the protein, and finding clusters of probes with the most favourable binding energy. These clusters are placed in rank order of the likelihood of being a binding site according to the sum total binding energies for each cluster (http://bioinformatics.leeds.ac.uk/qsitefinder/) (15).

Results and Discussion

Homology modeling:

In the results of CaR extracellular domain (residues 1 - 612) BLAST search against PDB, only three reference proteins, including extracellular region of the group II metabotropic glutamate receptor (16), metabotropic glutamate receptor subtype 1 (PDB ID: 1ewk) (17) and ligand-binding region of the group III metabotropic glutamate receptor (PDB ID: 2e4z) (16) had a good level of sequence identity and the identity of these three reference proteins with the CaR extracellular domain were 32%, 29% and 31% respectively.(Fig. 1) After doing multiple alignment (Fig. 2) by ClustalW program we used JALVIEW program to edit the alignment manually and due to lacking sufficient homology in resides 1-25 and 570-612 also because this residues did not include putative binding domain of this family according to Conserved Domain (CD) database in NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). (Fig. 3) Thus the model was made up of residues 26-569. Coordinates from the template

proteins were assigned to the target sequence based on the satisfaction of spatial restraints.. The initial model was thus generated with the above procedure. In this study, the DOPE potential profile in MODELLER was used to evaluate the model fold. The final structure was further checked by DOPE potential profile graphs. This energy profile graphs showed all residues had good energy profile except 330-350, 370-390 and 480-520 which were not satisfactory and hence considered for loop modeling. The same loops had a better profile in the template structures (data not shown), which strengthens the assessment that the model was probably incorrect in this loops. The loopmodel class in MODELLER software was used to refine regions of an existing coordinate file. This model was again evaluated and results showed that energy profile was improved. .(Fig. 4)

Evaluation of model:

After the refinement process, validation of the model was carried out using SWISS MODEL WORKSPACE using Ramachandran plot calculations computed with the PROCHECK program. The Φ and Ψ distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Tab1. (Fig. 5) Altogether 94.4% of the residues were in favored and allowed regions and VERIFY-3D environment profile was good and the results showed that this model is reliable. (Fig. 6)

Active site identification of CaR

After the final model was built, the possible binding sites of model were searched using the CASTp server, Pocket-Finding and Q-SiteFinder. According to comparison of this results and previous experiments which was showing the important rule of serine 170 and serine 147 in ligand binding (18) of this receptor, putative binding site in this protein is detected. (Fig7.)

This predicted 3-D model of extracellular region of calcium sensing receptor will be very useful in designing potent orthosteric agonists of CaR as new drugs.

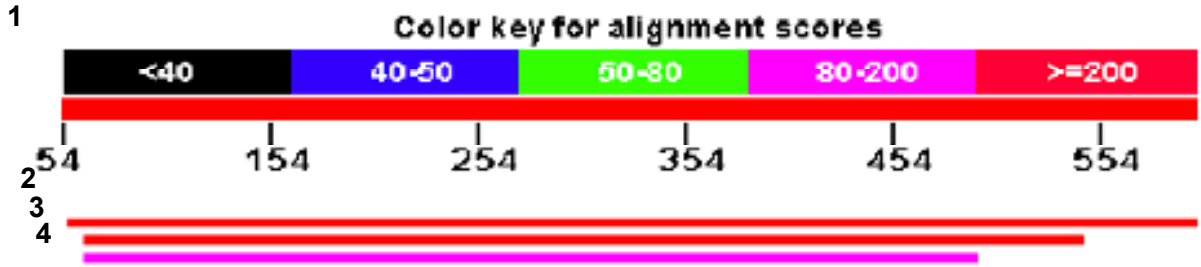

Figure 1. Blast output. 1) CaR extracellular domain 2)extracellular region of the group II metabotropic glutamate receptor 3) metabotropic glutamate receptor subtype 1 4)ligand-binding region of the group III metabotropic glutamate receptor.

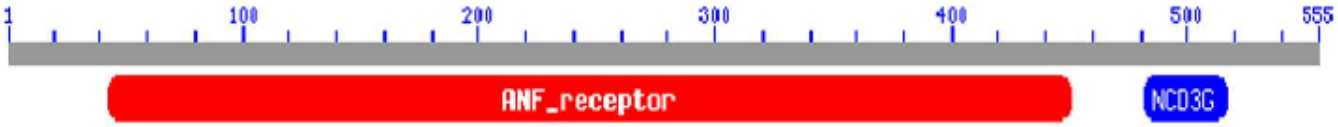

Figure 2. Conserved Dmain search output. Gray line is CaR sequence and Red box is conserved binding domain.

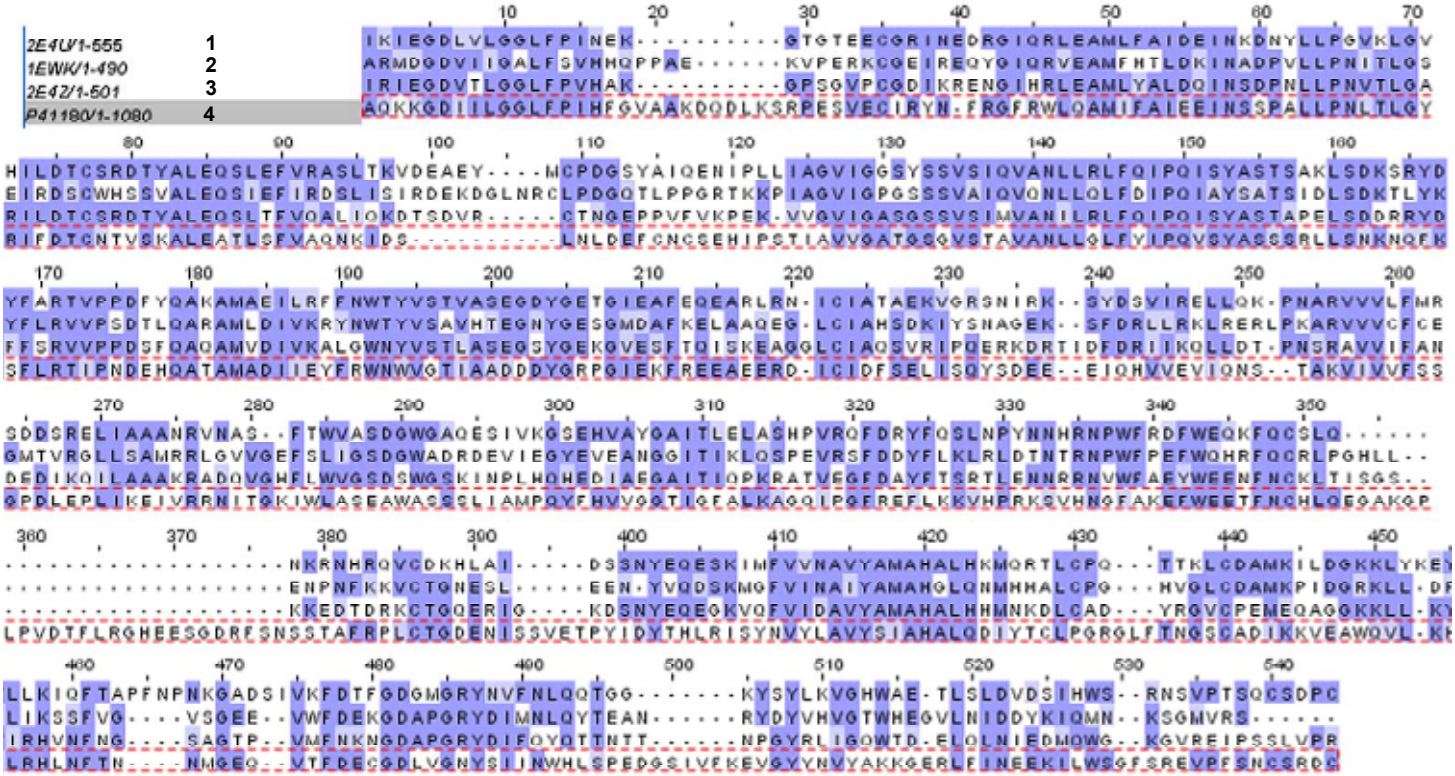

Figure 3. Multiple alignment result using ClustalW which is edited by JALVIEW. domain 1)extracellular region of the group II metabotropic glutamate receptor 2) metabotropic glutamate receptor subtype 1 3)ligand-binding region of the group III metabotropic glutamate receptor. 4) CaR extracellular domain

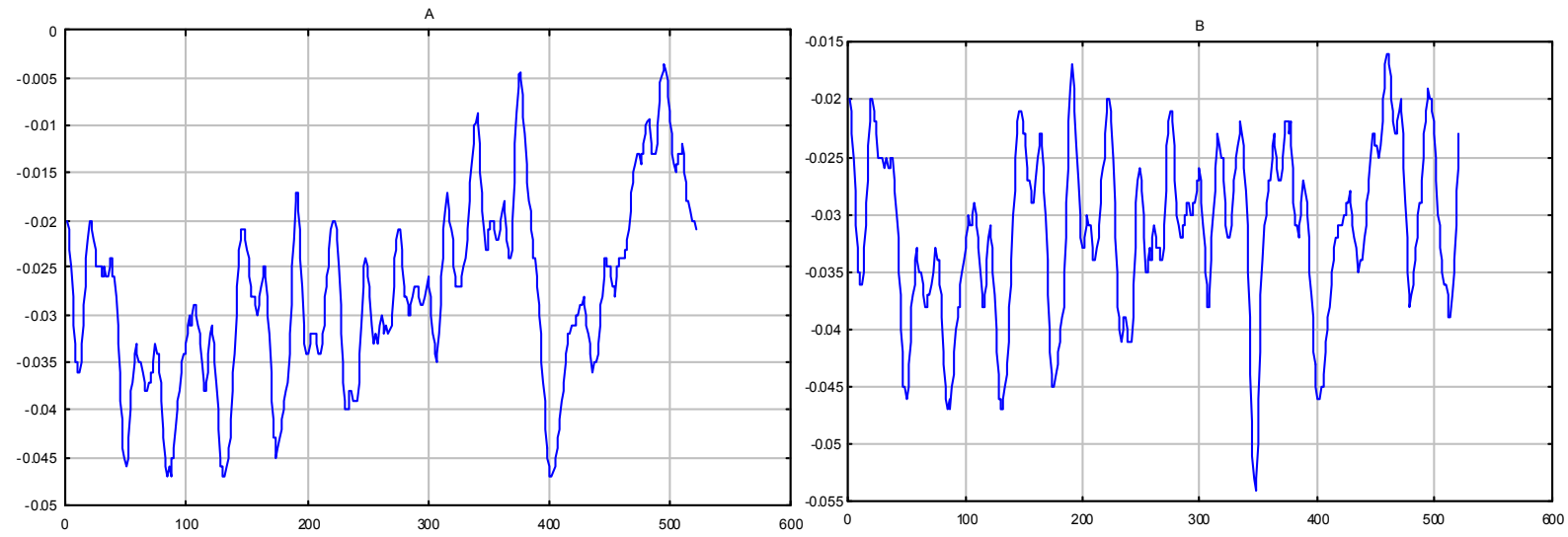

Figure 4. Energy profile of A) Rough model and B) refined model

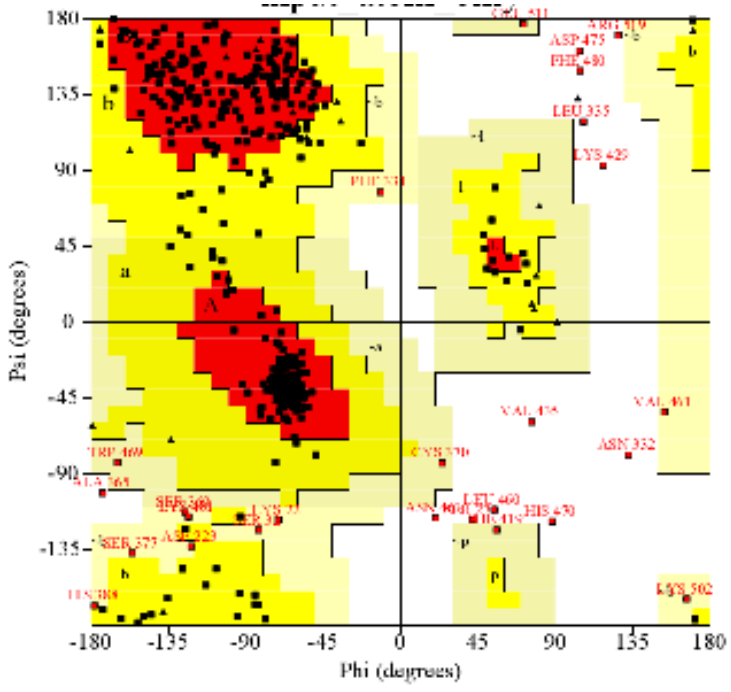

Figure 5. Ramachandran plot.

Figure 6.VERIFY-3D profile.

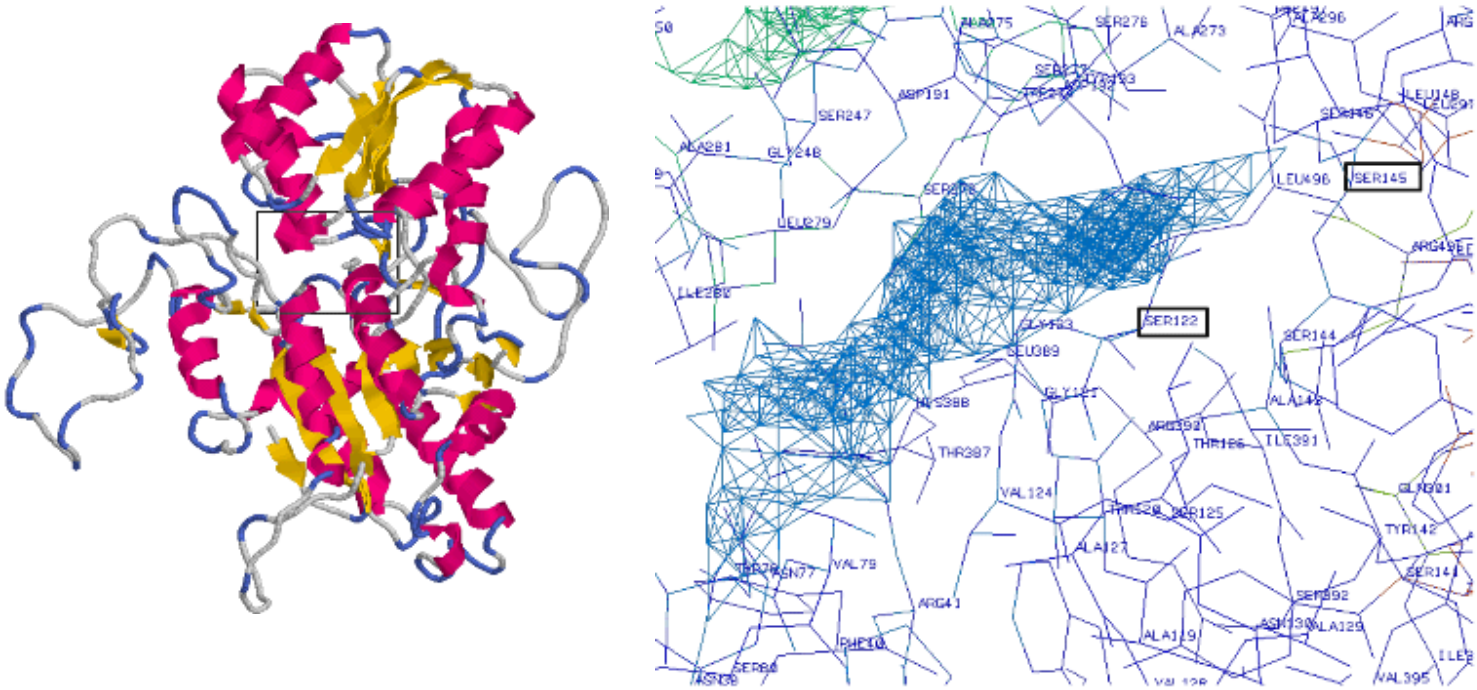

Figure 7. Left: 3D structure of CaR exteracellular domain and its binding site. Right: Putative binding site of CaR extracellular domain which interact with two important serine residues.

Table 1. Ramachandran plot results

	Number	Percent
Residues in most favoured regions	343	80.6
Residues in additional allowed regions	64	13.8
Residues in generously allowed regions	14	3.0
Residues in disallowed regions	12	2.6
Number of non-glycine and non-proline residues	463	100

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