Familial Hypercholesterolemia and Homology Modeling

Kasarah Allen
Frederick Douglass Academy
Dr. Yuying Gosser
Bioinformatics Workshop
City College of New York

Abstract

- Patients suffering from familial hypercholesterolemia have a much more serious condition than those people who have high levels of cholesterol. These FH patients have a much higher chance of suffering from heart attacks and stroke. Hypercholesterolemia describes the people with high levels of cholesterol. In other words, the people who have too much cholesterol circulating in their bloodstream. It is known to be caused by the consumption of a high-cholesterol diet and/or the genetic disease familial hypercholesterolemia (FH). Human body usually produces about two-thirds of its needed cholesterol in the liver, thus very little supplement of cholesterol is required. The homozygous FH is rarer, occurring with the frequency of about 1 in a million. The statistics for the homozygous FH is not surprising though; since patients suffering from two alleles of this gene usually do not survive pass their teens. The conditions of hypercholesterolemia in FH patients are detectable at birth or shortly thereafter. The cholesterol levels in heterozygous patients are between350 to 500 mg/d Patients suffering from familial hypercholesterolemia have a much more serious condition than those people who have high levels of cholesterol. These FH patients have a much higher chance of suffering from

heart attacks and stroke. Hypercholesterolemia describes the people with high levels of cholesterol. In other words, the people who have too much cholesterol circulating in their bloodstream. It is known to be caused by the consumption of a high-cholesterol diet and/or the genetic disease familial hypercholesterolemia (FH). Human body usually produces about two-thirds of its needed cholesterol in the liver, thus very little supplement of cholesterol is required. The homozygous FH is rarer, occurring with the frequency of about 1 in a million. The statistics for the homozygous FH is not surprising though; since patients suffering from two alleles of this gene usually do not survive pass their teens. The conditions of hypercholesterolemia in FH patients are detectable at birth or shortly thereafter. The cholesterol levels in heterozygous patients are between350 to 500 mg/dL, and in homozygous, the levels are between 700 to 1,200 mg/dL. Hypercholesterolemia is a silent disease. No symptoms will occur until the resulting chest pain of a heart attack or the symptoms of a stroke. Tendon xanthomas commonly occur in FH patients. The heterozygous will develop these later in life, but the homozygous usually develops them in their childhood. Xanthomas are lesions caused by cholesterol deposits in various parts of the body. The common places are in the extensor tendons of the hands and eyelids (know as xanthelasmas).

Introduction

Everyone knows that proteins play an important role in the research of a range of diseases. Proteins can give more insight to the origin or root of a particular disease. As we define and analyze the properties and characteristics of a protein the more information we know about the disease. By knowing the characteristics of a protein we can discover its involvement in the disease. Once we know how the protein affects the body we can begin to find ways to alter or eliminate the protein and ultimately finding a cure for the disease. Basically, proteins are the basis to finding cures to the present and future diseases of our time.

One way to reach that goal is threw structural analysis of proteins. For those proteins whose structures haven't been determined we use homology modeling. Homology modeling involves using a computer software program that determines the structure of the protein based on the protein sequence and the appropriate template. The computer software will do the modeling for us so that we are able to identify the properties of the protein and reveal the significance of the protein in a particular disease threw structural analysis.

The basis of homology modeling is based on the assumption that, if two structures have similar sequences then they will have similar structures. If the

sequence identity is greater than 90% then the predicted structure is practically the same as the template structure. If the sequence identity is lower than 25% then the predicted structure is not reliable. If the sequence identity is in the range 50%-80% then the homology modeling can generate reliable and informative structure. I used MOE (Molecular Operating Environment) to do the modeling; it does all the steps for you after the sequence is inputted.

One of the many rare diseases affecting 1 per 500 persons today is Familial Hypercholesterolemia; a common autosomal semi-dominant disease. Certain populations with Finnish, Lebanese, Ashkenazi Jewish, Afrikaner, or French Canadian origins have a higher prevalence of FH and the inheritance pattern is the same for both males and females. However males may suffer from severe hypercholesterolemia before women. There are two forms of familial hypercholesterolemia, heterozygous and homozygous. The gene responsible for this disease is the low-density lipoprotein receptor (LDL). The low-density lipoprotein receptor mediates cholesterol homeostasis through endocytosis of lipoproteins. The LDL receptor gene is located on the short arm of chromosome 19, and the protein is composed of 860 amino acids. Defects in LDLR are the cause of familial hypercholesterolemia. The receptor defect impairs the catabolism of LDL, and the resultant elevation in plasma LDL-cholesterol promotes deposition of cholesterol in the skin, tendons, and coronary arteries. Familial

Hypercholesterolemia is also associated with coronary artery disease and early detection and aggressive management to lower the LDL level helps to prevent or slow the progression of coronary atherosclerosis.

A major change in the number or functional status of LDL receptors directly affects plasma cholesterol levels. If the liver does not take up LDL particles, plasma LDLc levels increase. Also, when LDL is not internalized by the hepatocytes (large polyhedral epithelial cells invovled in protein synthesis), hepatic synthesis of cholesterol is not suppressed. This leads to further cholesterol production despite high levels of circulating cholesterol so circulating cholesterol levels are increased significantly. High levels of LDL cause enhanced cholesterol uptake in non-hepatic cells that is independent of LDL receptors. These pathways allow cholesterol uptake by monocytes (leukocyte) and macrophages, leading to foam cell formation, plaque deposition in the endothelium of coronary arteries, and premature coronary artery disease. Several conditions other than familial hypercholesterolemia cause severely elevated LDL levels, and each is caused by a single gene abnormality. LDL-receptor gene defects can be identified with genetic testing. Familial Hypercholesterolemia can be treated in many different ways, it depends on which form of familial hypercholesterolemia that you have. Since the LDLR protein doesn’t have a structure, it is my job to construct it.

Materials & Methods

The materials I used for developing the structure for the low-density lipoprotein receptor protein were the sequence for my protein from the NCBI website and a computer software program called MOE (molecular operating environment).

There are many software for homology modeling, the general procedure is:

- Search for the template based on sequence similarity using BLAST (an online software provided by the NCBI database)
- Select the candidate template to make sure the E-value of the sequence alignment is less than 10-4 and sequence identity is greater than 50%.
- Input the target sequence and the template sequence and structure into homology modeling software to do modeling, for example SWISS MODEL (***http://swissmodel.expasy.org/SWISS-MODEL.html***), or MOE.
- Check the accuracy of the generated structure model.

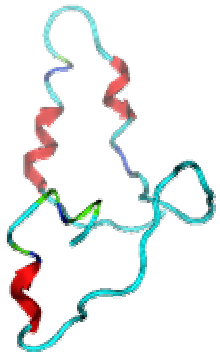
So after I got my sequence from the website

- I saved it in the FASTA format which is given by the website.

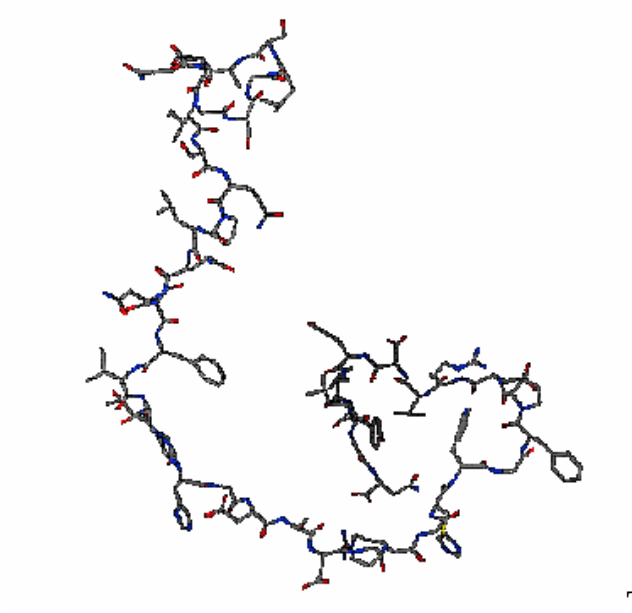
- I pasted the sequence into MOE and searched for the appropriate template.
- I loaded my template and sequence, also known as a query, and aligned the sequences.
- MOE then models the target protein structure based on the template. structure
- Then I verify the local geometry of the generated model by using a Ramachandran Plot.

Once you have your structure you can move it in any direction and examine it from all sides. You label the amino acids, the elements. You are also able to highlight specific points, places, or elements. You are also able to change the way the model is presented. You may want to show the space filling model or the ball-and-stick model of your protein. MOE allows you to observe your protein in many ways.

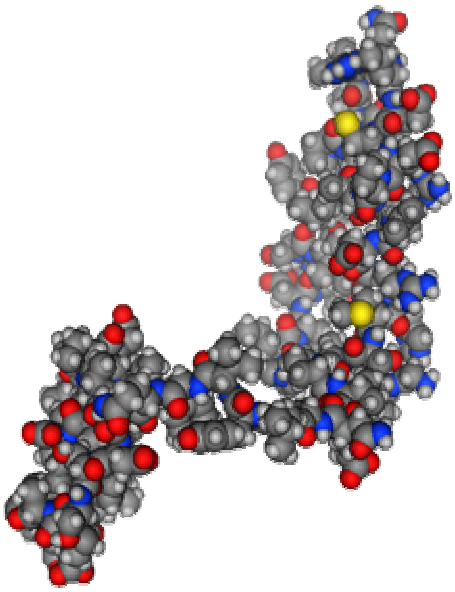
Results



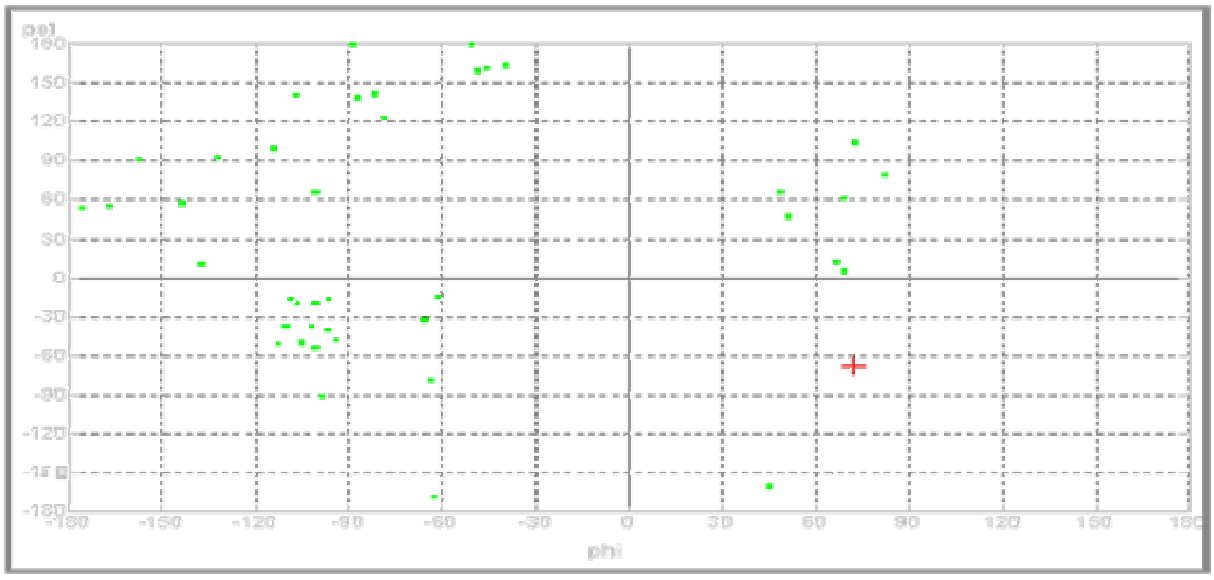
This is the backbone to the protein. It has some alpha helixes and little to no beta sheets. The red arrows are the alpha helixes and the green and blue strings are turns and loops.



This is the template to which I used to help generate the structure to my protein. It is a ball-and-stick model.



This is the space filling model of my protein. It shows the relative atomic sizes of the atoms of the molecule. The carbons are dark gray, the hydrogen's are light gray, the oxygen's are blue, the sulfur's are yellow and the carbon monoxides are red.



This is a ramachandran plot which also shows the alpha helixes and beta sheets and the geometry of the model.

Discussion

Curing homozygous patients is very difficult because they express little or no activity from the LDL receptor. They are resistant to most cholesterol-lowering drugs. Liver transplantation can provide the missing LDL receptors but requires special long-term follow-up cares for the transplanted organs, including continuous immunosuppressants. FH homozygous are currently treated with modified forms of plasmapheresis that selectively remove very small density lipoprotein and low-density lipoproteins from the plasma. Also, the modern approach to this problem is gene therapy. Heterozygous FH can be treated effectively with statins. These are drugs that inhibit the body's ability to produce cholesterol by blocking the enzyme hydroxymethylglutaryl CoA reductase (HMG-CoA-reductase), the rate-limiting enzyme in the cholesterol biosynthetic pathway. Statins work by forcing the liver to produce more LDL receptor to maintain the amount of cholesterol in the cell. There are many ways of treating Familial Hypercholesrerolemia, some of the other ways include drug therapy, diet change, excersise, etc. Drugs that can increase lipoprotein levels include aspirin, oral contraceptives, phenothiazines, corticosteroids, and sulfonamides. Exercise training has many cardiovascular benefits and can improve blood lipid levels. Predicting the degree of improvement in an individual's lipids levels with dietary change is difficult because many

variables affect the response, including the makeup of the baseline diet, the degree of patient compliance, and the individual's LDL responsiveness to the diet, which is genetically determined.

The predicted structure of my target protein helps me to analyze the importance of the protein and its association with Familial Hypercholesterolemia. Knowing the structure of the LDL receptor scientists can better understand the effects it has on Familial Hypercholesterolemia patients. For example, scientist can find why the LDL receptor fails to activate and bind to the surface and can learn how to provide LDL receptors to FH patients. In families with a history of familial hypercholesterolemia, genetic counseling is of benefit, especially if both parents are affected. Prevention of early heart attacks requires recognition of existing elevated LDL levels, and a low-cholesterol, low-saturated fat, high-unsaturated fat diet in high-risk people may help to control LDL levels.

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