

Molecular Modeling Studies: Exploring Potential Binding of Soy Protein Alpha Prime 7S Globulin Subunits to Human PCSK9 as a Mechanism for Enhancing LDL Receptor Recycling

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Introduction

The alpha prime region of the soybean 7S globulin subunit has been associated with a decrease in cholesterol both in *in vitro* and *in vivo* studies^{1,2}. One possible mechanism could be binding of the protein or its digested fragments to proteins associated with regulation of LDL receptor sequestering and recycling resulting in enhanced fusion of LDL receptor storage vesicles with the cell surface. Proprotein convertase subtilisin-like/kexin type 9 (PCSK9) is a serine protease that has recently been identified as an important regulator of LDL receptor levels.³ PCSK9 is thought to bind tightly to LDL receptors targeting them for transport and degradation in lysosomes. Inhibition of this interaction via mutations results in increased expression of LDL receptor and decreased LDL and cholesterol levels in both *in vitro* as well as *in vivo* studies.

In this paper we present the hypothesis that the observed decrease in cholesterol levels associated with the alpha prime region of the soybean 7S globulin subunit could arise from binding to PCSK9 resulting in decreased LDL receptor degradation and increased receptor recycling. In order to explore the feasibility of this hypothesis, molecular modeling experiments were performed using molecular modeling software (MOE, Chemical Computing Group, Inc.) and protein-ligand docking software (GOLD, Cambridge Crystallographic Data Centre).

The studies presented below are based on the x-ray crystal structure of wt-PCSK9 bound to wt-EGF-A of the LDL receptor (2W2M.pdb). Docking studies were first performed using wt-EGF-A to validate the docking methodology. Then docking studies were performed using a small active peptide from the alpha prime subunit, LRPAGTTFYVNPNDENLRMIA. Lovati and colleagues previously identified this peptide as having the ability to increase LDL receptor expression in hepatocytes.⁴

Materials and Methods

The x-ray crystal structure of wt-PCSK9 bound to wt-EGF-A of the LDL receptor (2W2M.pdb) was obtained from the PDB. To prepare the structure for docking and modeling studies, Deepview (SPDBV, GlaxoSmithKline) was used to excise the prodomain residues. The catalytic domain and EGF-A fragment were saved in separate *.pdb files and imported into MOE where the "Wash" function was used to refine the structures including addition of "explicit hydrogens".

To simplify the binding studies, the EGF-A structure was truncated to only include residues in contact with the binding site on the catalytic domain. This resulted in the following peptide fragment: NECLDNNGGCSHVCND. MOE was also used to generate the peptide LRPAGTTFYVNPNDENLRMIA. These structures were then exported for input into GOLD for docking.

Docking experiments were performed using the default GOLD fitness function evolutionary parameters. The carbonyl oxygen on Phe 379 (for 2W2M.pdb) was selected as the binding site centers for all calculations. Ten docking runs were performed per structure unless 3 of the 10 poses were within 1.5Å rmsd of each other. For the EGF-A peptide, key H-bonds identified from the x-ray structure were used as constraints.

Results

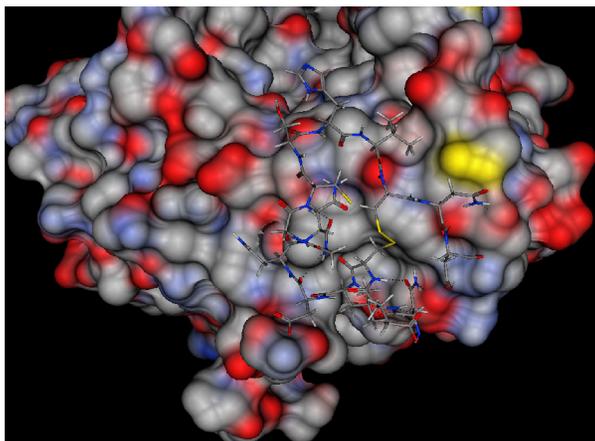


Figure 1. X-ray crystal of key contact residues of wt-EGF-A from LDL receptor (NECLDNNGGCSHVCND) bound to wt-PCSK9 (2W2M.pdb). Hydrogen bonds shown by dashed lines.

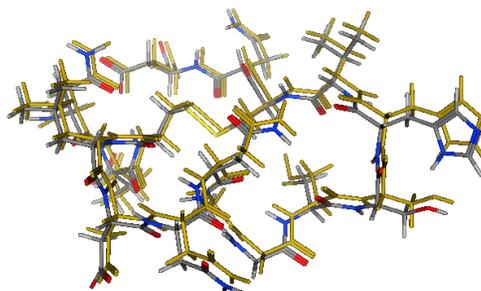


Figure 2. Superposition of top scoring docked pose of peptide fragment: NECLDNNGGCSHVCND (in gold) on truncated x-ray crystal structure of EGF-A (from 2W2M.pdb) in CPK, rmsd < 1 Å.

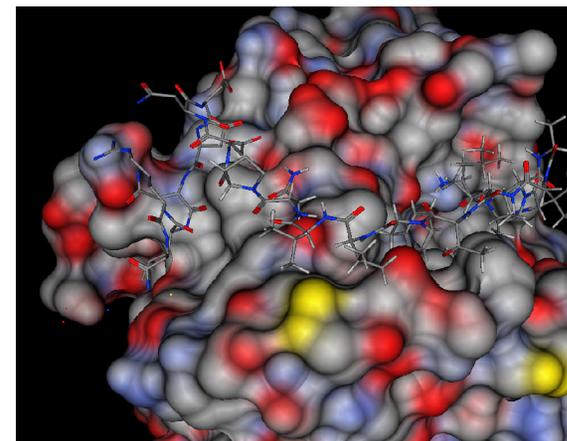


Figure 3. The top scoring pose of the peptide from 7S globulin alpha prime: LRPAGTTFYVNPNDENLRMIA docked to wt-PCSK9 (2W2M.pdb).

Discussion

In summary, molecular modeling and docking studies were performed in order to explore the possibility that the 7S Globulin alpha prime peptide from Glycine max could bind to the PCSK9 in lieu of the LDL receptor thus preventing degradation. The x-ray structure of the bound EGF-A peptide was truncated to the key contact residues (Figure 1), and docking studies were performed. The top scoring poses were well within rmsd < 1 Å of the x-ray conformation thus demonstrating the ability to model "correct" binding modes (Figure 2). Next we docked the peptide from 7S globulin alpha prime: LRPAGTTFYVNPNDENLRMIA (Figure 3) demonstrating that the peptide can bind to the same regions of PCSK9 as the EGF-A peptide of the LDL receptor.

Although the hypothesis presented in this paper is highly speculative, this computational-based study represents a first small step towards the generation of a structure-based mechanism of action for the well documented and observed affect of the soybean 7S globulin protein on cholesterol and lipid metabolism^{1,2,4}.

References

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