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New Binding Mode of SLURP Protein to α7 Nicotinic Acetylcholine Receptor Revealed by Computer Simulations

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SLURP-1 is a member of three-finger toxin-like proteins. Their characteristic feature is a set of three beta strands extruding from hydrophobic core stabilized by disulfide bonds. Each beta-strand carries a flexible loop, which is responsible for recognition. SLURP-1 was recently shown to act as an endogenous growth regulator of keratinocytes and tumor suppressor by reducing cell migration and invasion by antagonizing the pro-malignant effects of nicotine. This effect is achieved through allosteric interaction with α7 nicotinic acetylcholine receptors (alpha-7 nAChRs) in an antagonist-like manner. Moreover, this interaction is unaffected by several well-known agents specifically alpha-bungarotoxin.

In this work, we carry out the conformational analysis of the SLURP-1 by a microsecond-long full-atom explicit solvent molecular dynamics simulations followed by clustering, to identify representative states. To achieve this timescale we employed a GPU-accelerated version of GROMACS modeling package. To avoid human bias in clustering we used a non-parametric clustering algorithm Affinity Propagation adapted for biomolecules and HPC environments. Then, we applied protein-protein molecular docking of the ten most massive clusters to α7-nAChRs in order to test if structural variability can affect binding. Docking simulations revealed the unusual binding mode of one of the minor SLURP-1 conformations.

Keywords: molecular dynamics, gromacs, clustering, affinity propagation, protein docking, biomolecules.

Introduction

Three-finger proteins of the Ly6 family have multiple functions across the organism: from lignd-binding domains of growth factors receptors (myostatin receptor) to regulation of nicotinic receptor (nAChR) expression and function in the brain (Lynx1). Most mammalian Ly6 proteins have a GPI anchor at the C-terminus attaching them to the membrane while others do not have it and are secreted. Among the latter is SLURP-1 functioning as a water-soluble paracrine/autocrine messenger molecule which binds nicotinic acetylcholine receptors and regulates keratinocyte growth and angiogenesis. Such properties of SLURP-1 make it a valuable object to study as an endogenous cholinergic ligand similar to snake venom neurotoxins that played a crucial role in the nAChR research for decades [8, 10].

The purpose of the research was to find out alternative SLURP-1 complexes with nAChRs conformation patterns exist. Can there be different options for binding? It was previously shown that SLURP-1 does not compete with alpha-Bgt for the binding to α7 nAChRs. There were data, including NMR data, on the binding of SLURP-1 variants without N- and C-terminal tags and labels. But there is still no evidence about the binding mechanism. SLURP-1 fusion constructs bearing unnatural tags and labels show properties drastically different from those described for recombinant SLURP-1 which has only one additional N-terminal Met residue (PDB ID: 2MUO). Noteworthy, no experimental spatial structure of fusion SLURP-1 proteins

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were described. Fusion proteins demonstrated positive allosteric modulation of α7 nAChR while recombinant SLURP-1 act as an inhibitor of the receptor [8].

Since no competition with alpha-bungarotoxin is observed, some alternative binding sites of SLURP-1 should exist. In this work, the hypothesis was tested by molecular modeling methods. Molecular dynamics simulations were used to detect stable SLURP-1 conformations, and protein-protein docking was performed to identify prospective binding sites. In the course of the study, several characteristic patterns appeared that did not always correspond to NMR data. Changes in the secondary structure of one of the fingers were observed. Also, more than one variant of nACHRs binding model was found, and they depended on the conformation of SLURP.

Without experimental verification, it is difficult to determine the correctness of the prediction and unequivocally confirm or disprove the hypothesis.

1. Methods

Molecular dynamics simulation of SLURP-1 NMR conformation (1st model) (PDB ID: 2MUO) was performed in GROMACS [7]. Protein part was described with amber99sb-ildn forcefield [6] and explicit water model tip3p was used as a solvent. Computational resources of Lomonosov supercomputer in conjunction with GPU acceleration allowed us to reach total trajectory length of 2 mks.

The trajectory was clusterized with non-parametric clustering algorithm affinity propagation [5]. The secondary structure was predicted with DSSP [4, 9].

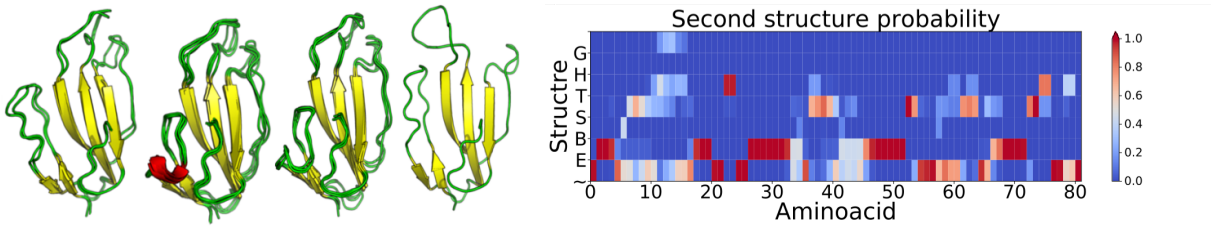
Protein-protein docking for 10 largest clusters was performed with ZDOCK [3] and replicated 10 times. The number of output complexes was set to 1000 and sorted by ZRANK. Top 10 hits from each replicate were selected producing 100 in total. Contacts at a distance less than 3.6 angstroms were calculated, and several models were selected for visual analysis with PyMol [2].

2. Results

2.1. Conformational Landscape of SLURP-1

During the visual analysis of the simulation of molecular dynamics, SLURP-1 underwent a change in the course of the backbone during the molecular dynamics toward the formation of the alpha helix like motif by the residues SER12, ALA13 and SER14. DSSP analysis of secondary structure over the whole trajectory confirmed formation 3-10 alpha helix for these residues Fig. 1 (a). Alpha helices are colored in red, beta-strands in yellow and unstructured areas in green. On Fig. 1 (b) the probability of participation of each amino acid in the formation of a certain secondary structure is presented, "$\sim$" – unstructured sections, "E" remains involved in the formation of beta strands, "B" – the situation where only one amino acid forms a hydrogen bond characteristic of the beta-strand, "S" and "T" – turns, "H" – alpha helix, "G" – 3-10 alpha helix.

With clustering analysis, 54 clusters were found and only 10 can be formally designated to two groups: with and without 3-10 alpha helix. Additionally, 10 replicas of the molecular dynamics simulation were performed for 100 nanoseconds each, showing reproducibility of the clusters.

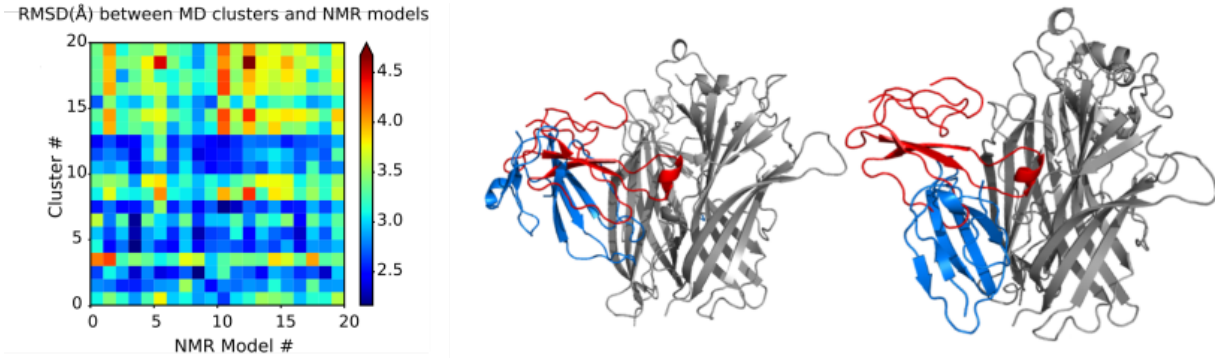


(a) The difference observed in loops and the presence or absence of a 3-10 alpha-helix site

(b) Secondary structure distribution over amino acids

Figure 1. SLURP-1 can form 3-10 alpha-helix by 12-17, amino acids and its loops dynamics

RMSD values were measured for all possible pairs of 20 simulation clusters and 20 NMR models Fig. 2. Remarkably, simulation data generally reproduced the conformation set from the NMR experiment.



(a) Comparison of MD clusters with NMR models

(b) Typical position of SLURP-1 (blue) and bungarotoxin (red)

(c) Atypical position of SLURP-1

Figure 2. Heatmap of MD clusters vs NMR models. Positions of SLURP-1 and bungarotoxin with two subunits of $\alpha 7$ nAChRs (grey)

2.2. Protein-Protein Docking of SLURP-1 to 7 nAChRs

Well-known ligand of $\alpha 7$ nAChRs receptor – alpha-bungarotoxin binds at the interface between subunits in pentamer. Results of protein docking of SLURP-1 conformations to $\alpha 7$ receptor presented two major binding modes. The first mode overlaps with the alpha-bungarotoxin binding site. Ligand interacts with the receptor by "fingers"– loop II and III regions without secondary structure involving residues 134-144 and 157-167 Fig. 2. The second binding mode does not overlap the alpha-bungarotoxin site, and SLURP-1 continued to have stable contact through the "fingers".

Disussion and Conclusions

According to simulation results SLURP-1 has two binding modes for various conformations. First binding mode overlaps with bungarotoxin site while the second one does not. Our data

provide clear directions for experimental support of newly proposed mode of interaction of SLURP-1 with α7 nAChRs.

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