IMPACT OF DYNAMIN-2 R465W MUTATION LINKED TO CENTRONUCLEAR MYOPATHY ON DYNAMIN INTERACTIONS, DYNAMIN DIMER, AND HELIX STRUCTURE: A STUDY USING MOLECULAR DYNAMICS SIMULATIONS

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FERNANDO ANDRES HINOSTROZA BALMACEDA

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Dirigida por: Dra. Ana María Cárdenas

Co-Dirigida por: Dr. Danilo González

FACULTAD DE CIENCIAS

UNIVERSIDAD DE VALPARAÍSO

INDEX OF CONTENTS

TABLE LIST

FIGURE LIST

SYMBOL LIST, ABBREVIATIONS OR NOMENCLATURE

AP: Adaptor protein.

atm: Atmosphere.

BSE: Bundle Signaling Element.

C: Cytosine.

ºC: Celsius degrees.

CG: Coarse-grained molecular dynamics.

CHARMM36: Chemistry at HARvard Macromolecular Mechanics 36.

CME: Clathrin-mediated endocytosis.

CNM: Centronuclear myopathy.

DNM1: Dynamin gene 1.

DNM2: Dynamin gene 2.

DNM3: Dynamin gene 3.

DOPE: Discrete Optimized Protein Energy.

Dyn-1: Dynamin-1.

Dyn-2: Dynamin-2.

Dyn-3: Dynamin-3.

fs: Femtoseconds.

G domain: GTPase domain.

GED: GTPase effector domain.

GMPPCP: guanosine 5’-[(beta, gamma)-methyleno] triphosphate.

GTP: Guanosine triphosphate.

HSC70: Heat shock cognate 70.

HT dimer: Heterodimer

HT helix: Hetero helix

K: Kelvin degrees.

LDL: Low density lipoprotein.

MD: Molecular dynamics simulations.

mM: milimolar.

MM/GBSA: Molecular Mechanics-Generalized-Born and Surface Area continuum solvation.

NaCl: Sodium chloride.

nm: nanometer.

NPT: Number of particles, pressure, and temperature.

ns: nanosecond.

NVT: Number of particles, volume, and temperature.

PC: Principal component.

PCA: Principal component analysis.

PDB ID: Protein Data Bank Identification Number.

PH: Pleckstrin homology.

PRD: Proline rich domain.

ps: picosecond.

R465W dimer: Mutant dimer.

R465W helix: Mutant helix.

RMSD: Root mean square deviation.

T: Thymidine.

μs: microsecond.

VMD: Visual molecular dynamics.

WT: Wild-type.

WT dimer: Wild-type dimer.

WT helix: Wild-type helix.

Centronuclear myopathy (CNM) is a dominant and debilitating disease. Patients with CNM exhibit progressive muscular weakness affecting distal skeletal muscles. Several mutations in the gene encoding for dynamin-2 (Dyn-2) causes CNM, being the most common mutation an arginine being replaced by a tryptophan at the position 465 (R465W). The most studied function of Dyn-2 is endocytosis, wherein this protein breaks the neck of the vesicle that is being endocytosed. To do this, Dyn-2 oligomerizes forming a helix, which binds and hydrolyzes GTP to get the energy needed to constrict and break the plasma membrane. It has been reported that the R465W mutant has an augmented GTPase activity and forms abnormally stable oligomers. However, how the R465W mutation impacts Dyn-2 structure is not completely understood.

To understand how this mutation affects Dyn-2 structure, I took advantage of full-atom molecular dynamics simulations, which allowed me to determine, at atomic level, how this mutation impacts Dyn-2 structure. Since the basic unit of the Dyn-2 helix is the Dyn-2 dimer and CNM is a dominant disease, three different dimer systems were built: a wild-type (WT) dimer, a hetero (HT) dimer composed of one WT and one mutated protein, and a mutant dimer (R465W dimer) consisting of two mutated Dyn-2. A 150 ns simulation was run for each system. The analysis revealed that mutation R465W: (1) changes the curvature of the stalk alpha-helices. (2) increases the number of interactions between monomers, and (3) generates more compact dimers. Importantly, the structural changes adopted by the mutant monomer are transmitted to WT dynamin in the HT dimer.

To determine how the R465W mutation impacts Dyn-2 helix at a molecular level, I used Coarse-grained molecular dynamics simulations (CG). In this method, four heavy atoms are represented as a single coarse-grained bead. Three helices were built: one composed of 24 WT proteins (WT helix), a HT helix consisting in 12 WTs and 12 mutant Dyn-2s placed at random, and a mutant helix (R465W helix) composed of 24 mutated proteins. A carbon nanotube was added to each Dyn-2 helix to avoid helix collapse during the simulation. Each system ran for 2.5 µs of simulation. The analysis showed that R465W mutation changes: (1) the helices' structures, being this alteration more significant in the HT helix than the R465W helix, and (2) the helices' motions. Regarding the latter, the WT helix tended to open during the simulation, whereas HT and R465W helices tended to close and constrict the tube. The analysis of the monomers that form part of the helices reveal that a dynamin region called bundle signaling element, a flexible domain of the protein that transmit assembly-dependent conformational changes between different dynamin regions, bends concerning the stalk. This latter conformational change was not only observed in the mutant proteins but also in the WT monomers.

Taking together these analysis reveal that the R465W mutation causes structural changes not only in the monomers containing the mutation but also on the WT monomers that form part of HT oligomeric structures. This could explain the dominance of the mutated protein in the autosomal dominant centronuclear myopathy caused by dynamin mutations.