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Research paper

On the interactions between nucleotide binding domains and membrane spanning domains in cystic fibrosis transmembrane regulator: A molecular dynamic study

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ABSTRACT

The Cystic Fibrosis Transmembrane Regulator (CFTR) is a membrane protein whose mutations cause cystic fibrosis, a lethal genetic disease. We performed a molecular dynamic (MD) study of the properties of the nucleotide binding domains (NBD) whose conformational changes, upon ATP binding, are the direct responsible of the gating mechanisms of CFTR. This study was done for the wild type (WT) CFTR and for the two most common mutations, ΔF508, that produces a traffic defect of the protein, and the mutation G551D, that causes a gating defect on CFTR. Using an homology model of the open channel conformation of the CFTR we thus introduced the mutations to the structure. Although the overall structures of the G551D and ΔF508 are quite well conserved, the NBD1-NBD2 interactions are severely modified in both mutants. NBD1 and NBD2 are indeed destabilized with a higher internal energy (E_i) in the ΔF508-CFTR. Differently, E_i does not change in the NBDs of G551D but, while the number of close contacts between NBD1 and NBD2 in ΔF508 is increased, a significant reduction of close contacts is found in the G551D mutated form. Hydrogen bonds formation between NBDs of the two mutated forms is also altered and it is slightly increased for the ΔF508, while are severely reduced in G551D. A consequent modification of the NBDs—ICLs interactions between residues involved in the transduction of the ATP binding and the channel gating is also registered. Indeed, while a major interaction is noticed between NBDs interface and ICL2 and ICL4 in the WT, this interaction is somehow altered in both mutated forms plausibly with effect on channel gating. Thus, single point mutations of the CFTR protein can reasonably results in channel gating defects due to alteration of the interaction mechanisms between the NBDs and NBDs—ICLs interfaces upon ATP-binding process.

1. Introduction

The cystic fibrosis transmembrane conductance regulator protein (CFTR) is an anion channel, whose mutations, including those that lead to misfolding and loss of channel activity, cause cystic fibrosis (CF). CFTR is a member of the ABC (ATP-Binding Cassette) transporter family. The architecture of CFTR consists of two transmembrane domains, each linked to a nucleotide-binding domain (NBD). These two motifs are connected by a regulatory domain (RD), which requires phosphorylation by Protein Kinase A (PKA) in order to allow the activation of the channel. The opening and closing of the channel (gating) are modulated by the association and hydrolysis of adenosine triphosphate (ATP) by the NBDs. This means that activation and gating of the channel involve conformational modifications in the intracellular part of the protein, while this latter modification provides the power stroke for opening and closing the channel.

Abbreviations: ATP, adenosine triphosphate; ABC, ATP-binding cassette; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; EM, electron microscopy; ICL, intracellular loop; LIE, linear integration of energy; MD, molecular dynamics; NBD, nucleotide binding domain; PKA, protein kinase A; PME, particle-mesh Ewald; RD, regulatory domain; RMSD, root mean square deviation; SAXS, small-angle x-ray scattering; SPME, smooth particle-mesh Ewald; WT, wild type.

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Little is known about the structure of the whole CFTR protein. Like many other mammalian membrane proteins, full-length CFTR has proven to be difficult to purify because of its low expression levels. Moreover it requires detergents to render the protein soluble in aqueous solutions. Electron microscopy (EM) of two-dimensional crystals and single-particle analysis have permitted to discriminate, at low resolution, two different conformational states probably representing the open and closed states of the channel [1,2]. Also, structural changes of the whole CFTR and recombinant RD upon phosphorylation have been studied by cryoelectron microscopy [3,4], and small-angle x-ray scattering (SAXS) [5]. Furthermore, this latter scattering technique was also proficient in the observation of NBDs conformational changes associated to ATP-binding [6,7]. Nevertheless, the only structural data available at atomic resolution of CFTR come from recombinant NBDs [8–10].

Several homology models of the full-length CFTR protein structure have been published (for a review see Ref. [11]). We have studied the properties of the nucleotide binding domains on the framework of the full-length CFTR open channel model proposed by Mornon and collaborators [12,13], based on the structure of the Sav1866 bacterial transporter crystal structure from *Staphylococcus aureus*. Applying Molecular Dynamic (MD) simulations, we analysed the properties of the NBD1/NBD2 interactions and the nucleotide binding to these domains. The study involved the Wild Type (WT) model, and two mutants, G551D and ΔF508. The former G551D mutation impairs the gating mechanism of CFTR, reducing dramatically the anion transport across the channel. Differently, the latter mutation is a faulty assembling and transport of the protein, that is degraded before to reach the plasma membrane. Several strategies have been proposed to overcome the defective CFTR transit, resulting in a significant release of the protein in the plasma membrane. However, the rescued ΔF508-CFTR present a gating defect that avoids from the correct functioning of the anion transport [14,15]. Our results show that, not only stability of conformations differ between WT and its mutated forms, as one can expect, but also their NBDs are destabilized. Besides this, the studied mutations modify the interactions between NBD1 and NBD2, as well as the interactions between NBDs and intracellular loops (ICL). However, distinctive modifications are produced by each mutation, suggesting a different mechanism that would result on a defective gating of the CFTR channel.

2. Methods

2.1. Molecular dynamics simulations of wild type and mutant CFTR models

We used the homology model of human CFTR, based on the SAV1866 template [12,13]. This model, in the outward-facing conformation, would correspond to the open conformation of the CFTR channel [12,13]. Some modifications were introduced on the original model to define an operative model, WT-CFTR, suitable for our MD studies and purposes. Because of the lack of an adequate template to model RD by homology [5,12,16], the prediction of its structure has a high uncertainty. Therefore, to avoid improper interactions between NBDs and RD, we removed this domain; i.e. we removed residues from 646 to 841. This can be justified by previous experiments that have shown that, after removal of this region, CFTR is still active and the ATP binding, hydrolysis and gating modulation processes, are still working [17–20]. Serine at position 422 was kept in the phosphorylated form, as is in the original homology model [12,13], and in agreement to the crystallographic structure of the NBD1 [10]. Finally, two ATP molecules were accommodated in the NBD1 and NBD2 composite sites.

Simulations were done with the program NAMD 2.9 [21] with the force-field CHARMM 27 [22,23]. After extensive minimization of the force-field energy, two new constructs were obtained introducing CF-mutations on the WT-CFTR model using the Mutator plug-in in the molecule visualisation program VMD [24]. The plug-in Mutator introduces a point mutation to the protein structure, finding the best rotamer for the lateral chain of the modified residue. The first mutation, G551D, was a substitution of glycine of the position 551 by aspartic acid. The second mutation, ΔF508, was a deletion of the phenylalanine in position 508. The new constructs, G551D-CFTR and ΔF508-CFTR, were again extensively minimized prior to proceed with the successive process. Noteworthy, we have not observed large differences between the NBD1 resulted from the minimisation of our construct and the crystallized ΔF508 NBD1 [8,9,25]. Subsequently, all three models were inserted into a lipid membrane and hydrated using the routines available in the program VMD. A 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer of 100 × 100 Å was used, while the two sides of the bilayer were solvated with standard TIP3 water molecules. Sodium and chloride ions corresponding to a concentration of 100 mM were included in the water phase to neutralize the charges of the system. The complete system, including the CFTR with bonded ATPs, the phospholipid bilayer, and water with ions, is represented in Fig. 1.

The system was extensively minimized by restraining protein heavy atoms and allowing water molecules to equilibrate the

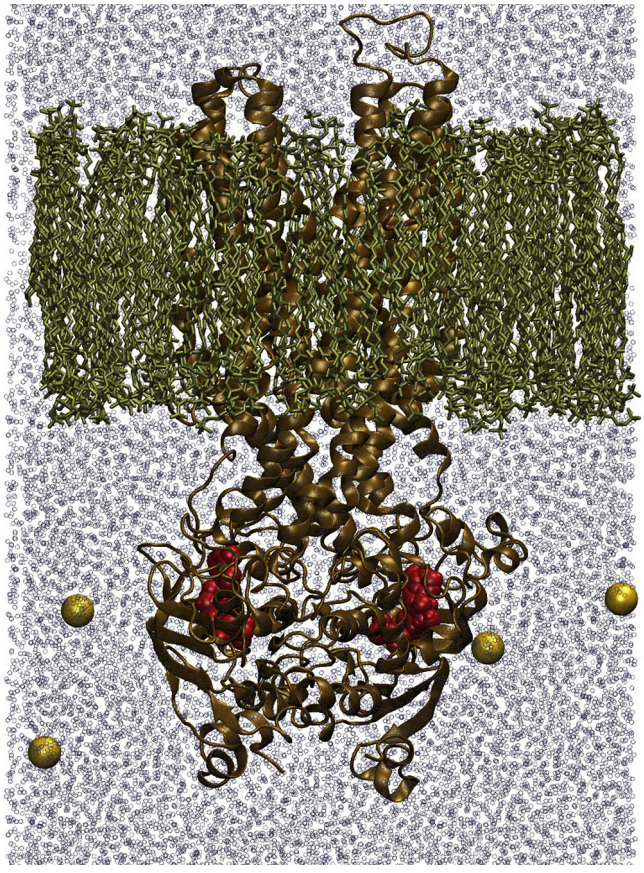

Fig. 1. The complete initial system used for the molecular modeling simulations. The model of the CFTR, in a cartoon representation, is inserted in the centre of a POPC bilayer depicted as tan licorice. The ATP molecules bound in the CFTR nucleotide binding domains are represented as red van der Waals spheres. Oxygen of water molecules at either side of the bilayer are depicted as ice blue dots, and ions are shown as orange spheres.