Genotype-phenotype correlations of myosin mutations: a molecular simulation study

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The primary cause of familial hypertrophic cardiomyopathy (FHC) has been attributed to mutations in the genes that encode the contractile proteins of the muscle cell. A majority of these mutations have been found in myosin, the principal component of the thick filament. Most in vitro studies have concluded that FHC mutations cause a loss of function in the biochemical and mechanical properties of myosin. Hypertrophy would then follow as a compensatory mechanism to raise the work and power output of the failing heart. Several recent studies, however, have thrown this mechanism into doubt, by providing evidence that FHC mutations in the myosin heavy chain (MHC) can enhance the functional properties of myosin. In trying to reach an answer to this problem, starting from homology modeling procedure, followed by intensive molecular dynamics simulations in water, we examined the structure and calculated the free energy of binding of myosin to ATP, in absence and\or presence of missense mutations, shown to be related to FHC.

Keywords: myosin, familial hypertrophic cardiomyopathy, missense mutations, free energy of binding

1 INTRODUCTION

Cardiomyopathies are diseases affecting the heart muscle, and are classified according to their anatomical and functional characteristics. The most common form is dilated cardiomyopathy (DCM), characterized by progressive dilatation and dysfunction of the cardiac chambers. The causes of the disease are mainly unknown, but it is now well recognized that genetic transmission of the disease occurs in many families (i.e., familial dilated cardiomyopathy, FDC), indicating the existence of a defective gene (or genes) as the cause of the disease in this subset of patients. The second most common form of cardiomyopathy is hypertrophic cardiomyopathy (HCM), which is characterized by primary cardiac hypertrophy. Like FDC, HCM is frequently inherited (FHC), and genetically heterogeneous. In the last ten years, molecular genetic studies [1,2] have revealed that mutations in genes encoding proteins of the sarcomere – such as β-myosin heavy chain (β-MHC), myosin binding protein C, cardiac troponin T, cardiac actin, α-tropomyosin and titin - can cause either DCM or HCM. The origin of this phenotypic heterogeneity, however, is still poorly understood. Cardiac β-myosin is an exameric protein consisting of two heavy chains (200 kDa each) (one shown in Figure 1), and two pairs of light chains, called essential light chain (ELC, 17 kDa) and regulatory light chain (RLC, 23 kDa).

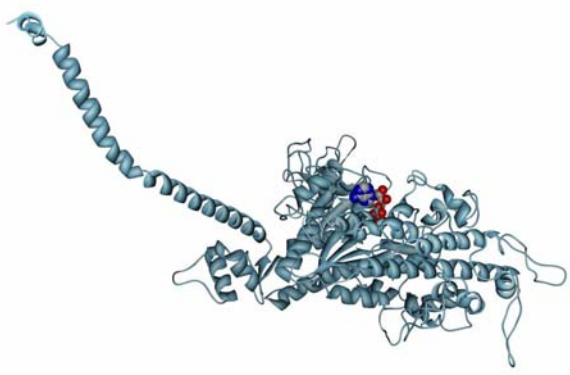

Fig. 1. β-myosin heavy chain. The ATP is highlighted in CPK representation.

The heavy chains are the molecular motor, which transduces free energy from the hydrolysis of ATP into movement and shortening of the sarcomere. Protein-nucleotide interactions give rise to conformational changes in the protein that result in the production of work. Despite concerted efforts to elucidate how the chemical free energy is harnessed, amplified, and transferred from the nucleotide binding site to the portion of the protein that functions as the working motor element, this process remains largely unresolved. Additional understanding of the protein-nucleotide interaction is necessary to resolve interaction, and is the goal we pursue in this work. Further, we will also test the hypothesis that different ATP binding site region mutations may have different effects on the functional properties of the protein, and in particular will focus on novel β-myosin mutations that may have different consequences on the myocardial function.

2 METHODS

2.1 Homology modeling

The first step in our procedure was to obtain a reasonable starting point for the 3D structure of the human β-myosin heavy chain, presently not available in the Protein Data Bank (PDB). Accordingly, we used the InsightII software (Accelrys Inc., USA), and its Biopolymer and Binding Site modules to generate a sequence alignment between the human and the highly homologous scallop myosin β-MHC Subsequently, the Modeler program [3] was employed, to build a preliminary 3D model of human β-MHC based on the alignment obtained in the previous step, and the 3D crystal structure of the scallop β-MHC reference protein. According to the followed method, the structurally conserved regions (SCRs) of the modeled protein are firstly identified and modeled, assigning the spatial coordinated directly from those of the references protein(s). The coordinates for the variable regions (the connections between these pre-built pieces), such as loops, are calculated either using the loop search procedure, that checks for similar pieces in the PDB data base or, when this region are confined to a few amino acids, using the replacement method. The quality of the final 3D homology structure is highly dependent upon the modeling procedure, and is influenced by a number of factors, the most important being sequences alignment. A too low value of the sequence alignment identity may lead to significant differences in the location of some residues. Using scallop β-MHC as reference protein (PDB entry 1QVI) ensured an exceedingly high value of 64% identity. The complete model structure obtained with our procedure was refined by several energy minimization rounds. Side chain positions were firstly optimized, keeping the protein backbone fixed. This constraint was then removed, and further protein relaxation was performed. The Amber software [4], with the Cornell et al. parameter set [5] was used to this purpose. The optimized 3D model was then used as the entry point for molecular dynamics (MD) simulations.

2.2 Molecular modeling

The second step was to study the structural and energetical features of the β-MHC ATP binding region with mutant residues. To confine calculations to reasonable CPU times, in the MD simulations we considered as flexible only a 20 Å subset of the of β-MHC:MgATP complex, centered on the ATP molecule. Each considered mutation was introduced to the wild type 3D model of the β-MHC:MgATP complex using the Biopolymer module of InsightII, by swapping the mutant residue into the specific site. Starting mutant side chain orientation was selected using the side chain rotamer library method. To asses how significant the replacement of a residue with its mutation was in terms of binding, we completed free energy calculations for MgATP as the nucleotide and β-myosin as receptor. The free energies of binding of the nucleotide to the enzyme were calculated using the snapshots from the corresponding MD simulations to yield the average molecular mechanical energy, (E_{MM}), the sum of the average nonpolar and polar solvation terms (G_{PBSA}), and the entropic term, $-T(S_{MM})$ [6]. The evaluation of the entropic contribution to the binding energy, is always very computationally intensive. In this particular case, due to the size of the systems considered, and to the fact that (i) the ligand was always ATP and (ii) the entropic variation due to a missense mutation is quite small, we decided to fix it to the value of +20 kcal /mol. This value was also seen to be appropriate in a study of ATP binding to kinesin-family motors [7].

3 RESULTS

3.1 Homology modeling

By virtue of the high sequence identity between reference and target protein, the SCR regions were quite extended; contemporarily, the loop regions were small, thus allowing for the use of the more accurate molecular replacement method.
The quality of the model was further assessed by using different validation tools. We performed praline puckering, packing quality, stereochemistry of main-chain and side-chain residues using the program PROCHECK. Ramachandran plot statistics indicated that 92% of the main-chain dihedral angles are found in the most favorable region, thus confirming the exceptional quality of the 3D model of human β-MHC obtained (see Figure 1).

3.2 Molecular modeling

Table 1 reports, as an example, the calculated free energy components for the ATP, the wild type β-MHC and the ATP:β-MHC complex, respectively.

Table 1. Energies of the β-MHC:ATP complex. All values are in kcal/mol.

Energy	Complex	β-MHC	ATP
E_{ele}	- 17496	- 16055	- 1064
E_{vdW}	- 5419	- 5404	+ 40
E_{int}	+ 9169	+ 9100	+ 87
E_{MM}	- 13746	- 12359	- 937
G_{np}	- 184	- 180	- 4
G_{PB}	- 10210	- 10210	- 408

Assuming $-T(S_{MM})$ as + 20 kcal/mol (see § 2.2), and adding this to the enthalpies given in Table 1, we found that ΔG_{bind} = - 22 kcal/mol for the β-MHC:ATP system. We note here that our calculated value is in excellent agreement with the experimentally obtained value of – 18 kcal/mol [8], and with the value of – 16 kcal/mol, obtained from other authors using the chicken β-myosin structure [9]. The highly negative value of ΔG_{bind} implies that the protein is expecting a nucleotide with a geometry more similar to a prehydrolysis nucleotide state (MgATP with tetrahedrally coordinated oxygens at the g-phosphate position) than to a transition state intermediate.
In analyzing the interactions between ATP and β-MHC, we can observe that there is a dense network of hydrogen bonds involving residues belonging to the P-loop, the switch 1 and the switch 2. Moreover, some water molecules seem to play an important role, as one coordinates with the Mg^{++} cation, one bridges a glutamic acid and a serine, and another hydrogen bonds an aspartic residue.
At the time of writing, several mutations that have been proven to be the cause of familiar hypertrophic cardiomyopathy are considered for simulations. We expect that the difference in the free energies of binding of the mutated isoforms of β:MHC with ATP will reflect the decreased affinities of these aberrant proteins towards the nucleotide. Moreover, the detailed analysis of the interactions of both wild-type and mutated myosins will allow to find a rationale for these clinical evidences.

4 CONCLUSIONS

The combination of homology and molecular modeling has resulted in a robust and convincing 3D structural model for human β-myosin heavy chain.
The calculations performed using the so-called Molecular Mechanics/Poisson-Boltzmann Surface Area approach has led to the obtainment of the value of – 22 kcal/mol for the free energy of binding of ATP and β-MHC, in excellent agreement with literature. Further, the combination the computational mutagenesis study of the ATP binding- site, currently in progress, will provide, for the first time, an important insight into β-myosin structure – function relationships.

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