The effect of Hypertrophic Cardiomyopathy – related β - myosin mutations on structural order of human muscle fibers and myofilaments

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Familial Hypertrophic Cardiomyopathy (FHC) is the most common genetic heart disease and the most frequent cause of sudden death in young people. FHC affects 1 in 500 individuals at all ages and is also an important cause of heart failure (1). To date about 500 different mutations in more than 18 different genes of mostly sarcomeric proteins involved in muscle contraction, are known (2, 3). Clinical manifestation of FHC is quite variable even among mutations affecting one specific protein. Molecular mechanisms of how mutations trigger the expression of the FHC phenotype are still poorly understood (4).

We study functional effects of FHC-related missense mutations in the heavy chain of β-cardiac myosin (β-MyHC) (i) to gain insight into the properties of structural subdomains of the myosin head at the molecular level and (ii) to identify disease inducing mechanisms (5, 6). For these studies we use muscle fibers from FHC patients with β-MyHC-mutations. One important question is whether myosin mutations affect the structural organization and order of the muscle thick filament (the myosin filament), which in turn also would influence force generating capabilities of the sarcomeres. It is currently unclear whether mutations in the head domain might affect filament assembly, as it has been suggested for mutations in the myosin rod (7). The best method to address this point is to record 2D-X-ray diffraction patterns of muscle fibers with these mutations. In the present study we examined whether FHC missense mutations Arg723Gly and Gly741Arg in the head domain of β-MyHC affect myosin based reflections like the meridional 14.3nm reflection (M3), off-meridional myosin layer lines (MLL) under relaxing conditions at different temperatures and the spacing of the innermost equatorial reflections (d₁₀). M3 corresponds to the repeat distance between the crowns of myosin heads along the myosin filament and thus reflects the helical order of the thick filaments. The myosin layer line intensities depend on the helical order and the conformation of the myosin heads (closed conformation induces strong MLL’s; 8). d₁₀ is the distance of the 1,0 plane of the equatorial (cross-sectional) lattice. It is calculated from the innermost equatorial reflections, and indicates the distance between the thick filaments.

Since in humans the β-MyHC is also expressed in slow skeletal muscle fibers, we used fibers isolated from M. soleus biopsies of FHC-patients with mutations Arg723Gly and Gly741Arg, respectively, for our studies. Arrays of some 30 single, chemically permeabilized fibers (9, 10) were prepared. 2D-X-ray diffraction patterns were recorded without nucleotide (rigor) at 2°C (Fig.1), and under relaxing conditions at different temperatures between 2°C and 30°C.

The resulting diffraction patterns showed no indication for structural changes. Fig. 1 shows diffraction patterns recorded in rigor. The M3-spacing is identical for mutant and control fibers, indicating the mutation R723G does not affect the packing and thus the helical order of the myosin filaments. The same was found for mutation G741R. Analysis of the equatorial reflection spacing revealed also no difference between both mutants and control fibers. This shows that also the filament lattice is not affected by the mutations. This reveals that the observed increase in active force generated per cross-sectional unit of muscle fibers is neither the result of different packing of myosin molecules within the myosin filaments nor of closer packing of myosin filaments within the contractile apparatus. Instead, the 2D-X-ray patterns imply that the increased force generation is
most likely due to increased force contribution of the individual myosin head domain. Analysis of MLL intensities at different temperatures is currently in progress, for statistical analysis additional experiments are necessary which will be performed in our next beamtime at A2.

Fig.1: 2D-X-ray diffraction patterns of muscle fibers from human *M. soleus* in the absence of nucleotide at 2°C. Left, fibers from healthy control individual. Right, fibers from patient with FHC mutation Arg723Gly. Patterns recorded at beamline A2, HASYLAB, exposure time 120sec, respectively. Note, that spacings of meridional myosin reflections M3 are the same for mutant and control.

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References