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Severe Myopathy Mutations Modify the Nanomechanics of Desmin Intermediate Filaments

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Mutations in the intermediate filament (IF) protein desmin cause severe forms of myofibrillar myopathy characterized by partial aggregation of the extrasarcomeric desmin cytoskeleton and structural disorganization of myofibrils. In contrast to prior expectations, we showed that some of the known disease-causing mutations, such as DesA360P, DesQ389P and DesD399Y, are assembly-competent and do allow formation of *bona fide* IFs *in vitro* and *in vivo*. We also previously demonstrated that atomic force microscopy can be employed to measure the tensile properties of single desmin IFs. Using the same approach on filaments formed by the aforementioned mutant desmins, we now observed two different nanomechanical behaviors: DesA360P exhibited tensile properties similar to that of wild-type desmin IFs, whereas DesQ389P and DesD399Y exhibited local variations in their tensile properties along the filament length. Based on these findings, we hypothesize that DesQ389P and DesD399Y may cause muscle disease by altering the specific biophysical properties of the desmin filaments, thereby compromising both its mechanosensing and mechanotransduction ability.

Keywords: atomic force microscopy; coiled-coil interactions; molecular slippage; desminopathy; myofibrillar myopathy

Introduction

Desmin is the major muscle-specific intermediate filament (IF) protein (for a review, see Ref. 1). Its sequence and expression pattern is evolutionarily well conserved from "primitive" fish to man.[2] In myocytes, desmin IFs form a well-ordered three-dimensional extrasarcomeric cytoskeletal network involved in several cellular functions such as maintenance of Z-disc registration, positioning of the nuclei and mitochondrial activity.[3,4] To date, more than 40 disease-causing mutations have been reported for the human desmin gene†. All these mutations are causing a distinct subgroup of myofibrillar myopathy, namely, desminopathy, which is characterized by disintegration of Z-discs and myofibrils as well as by the accumulation of desmin, αB-crystallin, plectin, ubiquitin and other proteins into large intracellular aggregates.[5] Patients become symptomatic around the second to third decade of life with affection of striated as well as smooth muscles.[5] However, it is cardiac involvement, which results in dilated, restrictive or even hypertrophic cardiomyopathy as well as cardiac arrhythmias,[6,7] that eventually causes death in these patients. The detailed molecular mechanism linking desmin mutations to the distinctive histological phenotype remains unclear. In *in vitro* assembly studies and in cell transfection studies, mutations in the desmin gene were found to lead to either (i) assembly incompetence, protein aggregation and segregation of mutant from wild-type desmin or (ii) the formation of mixed filamentous networks that seem very similar to those formed by wild-type desmin alone.[8] Surprisingly, most of the desmin mutants analyzed to date fall into the second category; that is, they exhibit a preserved assembly competence.[6,9] As both groups of desmin mutations ultimately lead to the same clinical and histopathological phenotype, this astounding finding calls for a better understanding of the potential impact of desmin mutations on structural and functional properties of the seemingly *bona fide* IFs formed by some desmin mutants.

Desmin, like other IF proteins, exhibits a tripartite secondary structure combining unfolded N- and C-

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† http://www.interfil.org

Abbreviations used: IF, intermediate filament; MPL, mass per length; AFM, atomic force microscopy; EM, electron microscopy; FWHM, full width at half maximum.

terminal domains with a double-stranded central α-helical coiled-coil domain interspersed in its middle by a non-α-helical flexible linker.[10] In this study, we have focused on the biophysical characterization of three filament-forming desmin mutants, all harboring point mutations located towards the C-terminus of desmin's central rod domain, namely, DesA360P, DesQ389P and DesD399Y.[8] The *in vitro* assembly properties of these mutants have been analyzed extensively and compared to that of wild-type desmin.[8,9] The assembly starter unit is a well-defined tetramer for DesA360P and wild-type desmin, whereas under identical buffer conditions, DesQ389P and DesD399Y form a heterogeneous population of larger complexes (Table 1).[9] Although *in vitro* all three mutants form filaments on their own and in combination with wild-type desmin, all three mutants were found to differ considerably from wild-type desmin with regard to filament radius and mass per length (MPL) (Table 1).[9]

In order to prove a potential disease-causing mechanism for these filament-forming mutants, we thought to seek alterations in their biophysical properties and to correlate those with the previously observed structural differences (Table 1).[9] To achieve this goal we have developed a single-filament approach based on atomic force microscopy (AFM).[11] This method allows the characterization of the tensile properties of single filaments adsorbed to a solid support. A first set of experiments with wild-type desmin filaments indicated an unusual extensibility up to 240% extension associated with an abrupt increase of the mechanical stress necessary to stretch the filaments above 50–100% extension, a phenomenon also known as "strain hardening" or "strain stiffening".[12] This behavior was associated with a strong decrease of filament diameter upon stretching, as observed by AFM imaging of single stretched filaments and by electron microscopy (EM) of sheared filamentous networks.[12]

In this study, we now employed the same approach to investigate the desmin mutants described above (Table 1) and demonstrate that these mutants could be separated in two categories according to their tensile properties. Filaments formed by DesA360P behaved similarly to filaments from wild-type desmin, whereas filaments formed by DesQ389P and DesD399Y exhibited heterogeneous tensile properties along the length of individual filaments. Interestingly, this mechanical heterogeneity that we now observed at the single-filament level correlates with the fact that DesQ389P and DesD399Y form a heterogeneous population of assembly starter units that assemble into irregular filaments with segments of different widths and heights (Table 1).[9]

Results

Morphology of mutant desmin filaments

At protein concentrations around 0.1 mg/ml used for previous EM investigations,[9] the three mutants analyzed in this study formed extensive networks that did not adsorb well on the mica substrates used for AFM. However, by lowering the protein concentration to 0.05 mg/ml, we obtained populations of either short or aggregated mutant desmin filaments on the substrate (Fig. 1a–c). The former were suitable for subsequent nanomechanical analysis.

DesA360P filaments exhibited a smooth height profile. However, they appeared knotted and some even ring-shaped (Fig. 1a, arrowheads and arrows, respectively). In contrast, filaments formed by DesQ389P and to a lesser extent by DesD399Y appeared irregular with segments of different widths and heights (Figs. 1b and c and 2a and b). The average height H_0 and the average full width at half maximum (FWHM) values for the different variants are compared to corresponding values for wild-type desmin filaments imaged under the same conditions (Table 1).[12] Among the mutants analyzed, only filaments formed by DesA360P exhibited an average height significantly higher than that of wild-type desmin filaments, corresponding well to the average radius R_0 as previously estimated by scanning transmission EM (STEM).[9]

Tensile behavior of mutant desmin filaments

We used the AFM tip to push laterally on single filaments.[12] For the three mutant desmin variants, we performed a total of 312 lateral pushing experiments with a vertical tip force fixed between 4 and 8 nN. In

Table 1. Structural parameters of desmin filaments

Desmin	Wild type	DesA360P	DesQ389P	DesD399Y
Assembly starter units[a]	Tetramers (A_{11})	Tetramers (A_{11})	Octamers and 12-mers[b]	Octamers and 12-mers[b]
R_0 (nm)[c]	6.3±0.65	7.25±0.65	5.6±0.85	5.7±0.45
MPL (kDa/nm)[d]	59±11	70±11	45±12	42±14
Height (nm)[e]	4.5±0.8 (n=60)	6.7±2 (n=79)	5±1.1 (n=40)	5.1±1 (n=26)
FWHM (nm)[f]	45±15 (n=60)	60±10 (n=79)	40±10 (n=40)	50±10 (n=26)

[a] Assembly starter units formed by the proteins after refolding in 5 mM Tris–HCl, pH 8.4, 1 mM DTT as assessed by analytical ultracentifugation.[9]
[b] These assembly starter units were longer than typical A_{11} tetramers, indicating the involvement of A_{12} and A_{22} interaction modes.[9]
[c] Average radius of the filaments estimated from STEM data.[9]
[d] Mass per length of the filaments estimated from STEM data.[9]
[e] Average height of the filaments measured by AFM. Data for wild-type desmin were taken from a previous study where the protein was analyzed under the same conditions.[12]
[f] Average FWHM measured by AFM. Data for wild-type desmin were taken from a previous study.[12]