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Impact of Disease Mutations on the Desmin Filament Assembly Process

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It has been documented that mutations in the human desmin gene lead to a severe type of myofibrillar myopathy, termed more specifically desminopathy, which affects cardiac and skeletal as well as smooth muscle. We showed recently that 14 recombinant versions of these disease-causing desmin variants, all involving single amino acid substitutions in the α-helical rod domain, interfere with *in vitro* filament formation at distinct stages of the assembly process. We now provide mechanistic details of how these mutations affect the filament assembly process by employing analytical ultracentrifugation, time-lapse electron microscopy of negatively stained and glycerol-sprayed/low-angle rotary metal-shadowed samples, quantitative scanning transmission electron microscopy, and viscometric studies. In particular, the soluble assembly intermediates of two of the mutated proteins exhibit unusually high *s*-values, compatible with octamers and other higher-order complexes. Moreover, several of the six filament-forming mutant variants deviated considerably from wild-type desmin with respect to their filament diameters and mass-per-length values. In the heteropolymeric situation with wild-type desmin, four of the mutant variants caused a pronounced "hyper-assembly", when assayed by viscometry. This indicates that the various mutations may cause abortion of filament formation by the mutant protein at distinct stages, and that some of them interfere severely with the assembly of wild-type desmin. Taken together, our findings provide novel insights into the basic intermediate filament assembly mechanisms and offer clues as to how amino acid changes within the desmin rod domain may interfere with the normal structural organization of the muscle cytoskeleton, eventually leading to desminopathy.

Keywords: intermediate filament; analytical ultracentrifugation; inherited mutations; myofibrillar myopathy; desminopathy

Introduction

The number of mutations in intermediate filament (IF) genes that have been shown to cause severe disease in man is increasing dramatically. Due to the tissue-specificity of IF expression, the phenotype in affected patients varies from blistering skin diseases in keratinopathies to premature ageing in laminopathies.[1,2] In muscle, mutations in the desmin gene can lead to cardiac and skeletal myopathy, termed desminopathy.[3]

Like all IF proteins, desmin exhibits a tripartite structure consisting of an amphipathic central α-helical "rod" domain flanked by non-α-helical end domains, i.e. an amino-terminal head and a carboxy-terminal tail domain. With the exception of a few missense mutations residing in either the head or the tail domain, most pathogenic desmin mutations discovered to date reside in the evolutionarily highly conserved coil 2B segment of the rod domain.[3–5] As yet, the impact of the wide variety of different desmin mutations on the filament assembly process is only poorly understood. Nevertheless, it has been hypothesized that these mutations generally abolish

Abbreviations used: IF, intermediate filament; STEM, scanning transmission electron microscopy; ULF, unit-length filament; MPL, mass per length; FWHM, full width at half maximum mass.

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filament formation and thereby yield characteristic desmin aggregates in muscle tissue.[4] In order to further our understanding of the pathogenic potential of desmin mutations for the development of desminopathy, we have performed a systematic analysis of 14 missense mutations located in the desmin α-helical rod domain.[6] The various mutations affect filament formation at distinct stages of the assembly process, thus enabling us to group them into four major categories: (1) apparently normal filament formation; (2) disturbed longitudinal annealing and radial compaction; (3) formation of filamentous aggregates; and (4) disintegration of assembly intermediates into globular aggregates. However, dissimilarities were noted even within a given group, particularly for mutant proteins that were still competent to form filaments. This situation calls for a more quantitative description of filament assembly, morphology, molecular architecture, and biophysical properties.

Driven by this need, we have employed a rigorous combination of analytical ultracentrifugation, time-lapse electron microscopy of negatively stained and glycerol-sprayed/low-angle rotary metal-shadowed samples, quantitative scanning transmission electron microscopy (STEM), and viscometric studies to obtain mechanistic details of how these desminopathy mutations may affect the filament assembly process. We observed distinct differences in the assembly kinetics and/or a severely transformed architectural design of the assembly products for most of the mutant desmin variants. Moreover, to better understand the influence of the mutated proteins on wild-type desmin (*Des*WT) assembly, we have assayed assembly mixtures of *Des*WT and each mutant variant by electron microscopy and viscometry. These latter experiments should provide more insight into the physiological situation with patients where wild-type and mutant protein are co-expressed, since nearly all mutations are inherited in an autosomal dominant manner.

Results

We employed recombinant murine desmin, which is 98% identical with human desmin and differs in only 3 out of 101 amino acids in coil 1B and 1 out of 121 amino acids in coil 2B. The primary reason for choosing murine desmin was to establish an experimental platform for investigating human desminopathy in transgenic mouse models. In support of this, our findings with mouse desmin are fully comparable to those obtained for human desmin, as the analysis of three homologous recombinant human desmin mutants (*Des*R350P, *Des*D399Y and *Des*R406W) yielded identical results (data not shown).

Complex formation of mutant desmin variants

Recombinant mutant desmin proteins were renatured from 9.5 M urea by dialysis into a low ionic strength Tris buffer (5 mM Tris–HCl (pH 8.4), 1 mM EDTA, 0.1 mM EGTA, 1 mM DTT).[7,8] Complex formation of each mutant desmin variant was then studied relative to recombinant *Des*WT by sedimentation velocity ultracentrifugation. As documented in Figure 1(a), *Des*WT and 12 of the 14 mutant variants analysed sedimented as homogenous species with *s*-values ranging between 4.8 S and 5.7 S (mean 5.2 S, Table 1). Remarkably, the mutant desmin variants *Des*Q389P and *Des*D399Y exhibited significantly higher *s*-values with peaks ranging from 6 S to 16 S for *Des*Q389P and from 6 S to 20 S for *Des*D399Y) (Figure 1(a)). Peak values obtained with two independent protein preparations were 9.5 S and 10.6 S for *Des*Q389P, and 11.9 S and 12.7 S for *Des*D399Y (Table 1). The broadening of the sedimentation curves indicates that heterogeneous populations of higher-order complexes had formed in both cases.

To gain more insight into the molecular nature of these higher-order complexes, we performed glycerol spraying/rotary metal-shadowing of *Des*WT and the two mutants. The complexes formed by *Des*WT exhibit a length of 50 nm–75 nm, corresponding to antiparallel, half-staggered tetramers (Figure 1(b), WT). This type of association is consistent with an *s*-value of ~5.2 S, as determined for tetrameric vimentin.[9] In contrast, both *Des*Q389P and *Des*D399Y yielded more complex associations: for *Des*Q389P various short filamentous species with a highly irregular diameter were seen (Figure 1(b)), whereas for *Des*D399Y comparatively short (50 nm–75 nm), thick aggregates were observed (Figure 1(b)). Considering their dimensions, these oligomeric species probably result from longitudinal (*Des*Q389P) or lateral (*Des*D399Y) annealing of tetrameric assemblies.

Mass per length determination of filament-forming desmin variants

At early assembly stages (i.e. 10 s), both *Des*Q389P and *Des*D399Y already formed long filamentous assemblies with hardly any residual unit-length filament (ULF), in contrast to *Des*WT, *Des*A213 V, *Des*E245D, *Des*A360P and *Des*N393I, which yielded predominantly ULFs (data not shown). This rapid filament assembly behaviour further complements the pronounced differences shown by these two mutant desmin variants on analytical ultracentrifugation and observed by glycerol spraying/rotary metal-shadowing electron microscopy under low-salt/high-pH conditions. The observation that the *Des*Q389P and *Des*D399Y mutant variants assemble much more rapidly into extended filamentous structures than *Des*WT protein suggests that these mutations may lead to a "relaxed" accuracy of some of the dimer–dimer interactions within the filament and thus to a more heterogeneous filament appearance. This is in accordance with the fact that *Des*WT ULFs exhibited a much more regular and distinct morphology than the highly irregular