A STRUCTURAL AND FUNCTIONAL INVESTIGATION INTO THE FILAMIN A G2593E MUTATION: IMPLICATIONS FOR NEUROLOGICAL DISEASE

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MANICKAVASAGAR ANANDASAYANAN

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ABSTRACT

The dimeric F-actin cross-linking protein human filamin a (hsFLNa) is an actin binding protein, which modulates the properties of the actin cytoskeleton and plays a major role in maintaining the integrity of plasma membrane associated actin, the viscoelastic properties of the cytoplasm, endocytosis, cytoplasmic streaming, cell division and cell motility [McGough *et al*, 1998; Small *et al*, 2002; Popowicz *et al*, 2006]. Dimerization is crucial for actin cross-linking functions of filamins [Davies *et al*, 1980] and the most C-terminal repeat of hsFLNa (hsFLNa24) is sufficient for hsFLNa dimerization [Himmel *et al*, 2003].

Several mutations in *hsflnA* are associated with pathologies such as periventricular nodular heterotopia (PVNH) [Robertson, 2005]. In this study we examined the possible cause and effect of a G2593E mutation in hsFLNa24 in a male patient diagnosed with PVNH on protein functionality. This was done by comparing relevant biochemical properties of wildtype and mutant hsFLNa24 proteins. For this purpose, recombinant proteins were expressed from cloned *hsflnA*24 coding sequence.

Full length wildtype *hsflnA24* (wt *hsflnA24*) was amplified from a cDNA library prepared from the PVNH patient. wt *hsflnA24* was used as the template to generate mutant *hsflnA24* (mt *hsflna24*) by site directed mutagenesis. The amplified wildtype and mutant sequences were cloned and over-expressed in an *Eschericia coli* system. hsFLNa24 proteins were isolated from crude protein extracts by immobilized metal affinity chromatography, purified by gel filtration and concentrated.

Several features of the mutant protein indicate that it has a high-entropy, disordered structure. Thermal stability of the two proteins determined by melt curve analyses showed that mt hsFLNa24 is less stable than wt hsFLNa24, their respective melting temperatures being < 303 K and 317 K, respectively. The mutant protein also tended to aggregate during concentration and was prone to precipitation during low speed centrifugation.

The two proteins displayed different elution volumes in size exclusion chromatography; wt hsFLNa24 eluted at a volume characteristic of a dimer indicating that in its native

form wt hsFLNa24 exists as a dimer, while the mutant's elution volume suggests that in most probabilities it exists as a monomer with a slightly larger molecular size. The oligomerisation status of the proteins in solution was further confirmed in crosslinking assays using the chemical crosslinker ethylene glycol succinimido succinate ester (EGS). In assays where the proteins at a final concentration of 4 μM were reacted with 1.3 mM EGS, the major form of wt FLNa24 was shown to be a high molecular weight dimeric species, while dimerisation was inhibited in mt hsFLNa24 and the protein exists predominantly in a monomeric state.

Both proteins were subject to various crystallizing conditions. Only wt hsFLNa24 produced diffracting crystals which have immunoglobulin (Ig)-like folds of the E-set superfamily [Murzin *et al*, 1995] with a predominantly β–sheet structure, where seven β-strands organized as two anti-parallel β–sheets of four (ABED) and three (CFG) strands, respectively, are arranged as a β-sandwich. The asymmetric unit consists of a dimer.

The dimer interface is formed by β-strands C and D of the monomers and the G2593 residue occurs in β-strand-C; it resides within the hydrophobic core of the dimer interface, where it is involved in a putative hydrophobic stacking. This glycine residue is highly conserved in most vertebrate filamins. Ablation of the G residue in conjunction with substitution by a polar residue is predicted to play a major role in disrupting the hydrophobic nature of the interface thereby inhibiting dimerisation. The inhibition effect arose probably more through unfavourable entropy change induced by substitution of the native G by an E residue than a reorganization of the dimer interface.

The actin cross-linking ability of filamins is ascribed to its dimerization mediated by the C-terminal repeats and the loss of the latter function may have serious implications to the patient harbouring the G2593E mutation. The relatively mild symptoms exhibited by the patient leads one to believe that there may be compensatory mechanisms in operation or that the basic premise that the most C-terminal repeat is important for filamin dimerisation is questionable.

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