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# The spectrin family of proteins: A unique coiled-coil fold for various molecular surface properties

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ABSTRACT

The spectrin superfamily is composed of proteins involved in cytolinker functions. Their main structural feature is a large central subdomain with numerous repeats folded in triple helical coiled-coils. Their similarity of sequence was considered to be low without detailed quantification of the intra- and intermolecular levels. Among the superfamily, we considered as essential to propose an overview of the surface properties of all the repeats of the five proteins of the spectrin family, namely α- and β-spectrins, α-actinin, dystrophin and utrophin. Therefore, the aim of this work was to obtain a quantitative comparison of all the repeats at both the primary sequence and the three-dimensional levels. For that purpose, we applied homology modelling methods to obtain structural models for successive and overlapping tandem repeats of the human erythrocyte α- and β-spectrins and utrophin, as previously undertaken for dystrophin, and we used the known structure of α-actinin. The matrix calculation of the pairwise similarities of all the repeat sequences and the electrostatic and hydrophobic surface properties throughout the protein family support the view that spectrins and α-actinin on one hand and utrophin and dystrophin on the other hand share some structural similarities, but a detailed molecular characterisation highlights substantial differences. The repeats within the family are far from identical, which is consistent with their multiple interactions with different cellular partners, including proteins and membrane lipids.


## 1. Introduction

The proteins of the spectrin superfamily contain a calponin type actin binding domain at their N-termini followed by multiple spectrin repeats that could specify an actin crosslinking characteristic distance. The superfamily includes plakins, nesprins, plectins, α- and β-spectrin, α-actinin, dystrophin and utrophin (Jefferson et al., 2007). This superfamily of proteins is characterised by the presence of multiple copies of spectrin-like repeats arranged in tandem (Parry et al., 1992; Baines 2003; Jefferson et al., 2007). The spectrin-like repeats (or repeats) are composed of three alpha-helices (A–C) wrapped into a coiled-coil. In the present study, we focused among the superfamily on the five proteins members belonging to the so-called spectrin family, namely α- and β-spectrin, α-actinin, dystrophin and utrophin. The first two proteins can form dimers/tetramers while the last two remain as monomers (Broderick and Winder, 2005).

Although the sequence similarity of the repeats among the spectrin family has not been studied quantitatively in detail, it is considered to be rather low (Winder et al., 1995). The unique feature of this coiled-coil structure is that the residues of the primary sequence are distributed in heptads, i.e., on seven successive residues (a–g), where the two residues in the 'a' and 'd' positions are hydrophobic while the others are mainly hydrophilic. The coiled-coil folding of the repeats is principally maintained by the heptad hydrophobic residues, buried away from the molecular surface and being in close contact. Therefore, the accessible residues of the repeats are mostly hydrophilic and situated in positions other than 'a' and 'd' (Parry et al., 1992; Lupas, 1996). This structural characteristic was demonstrated by the first crystals of spectrin solved by X-ray diffraction in 1993 (Yan et al., 1993) and more recently for the dystrophin and utrophin single repeat 1 (Muthu et al., 2012).

Two repeats are linked to each other to compose a filament consisting of a long common helix formed by the third helix of the first repeat and the first helix of the second repeat. This linking has been solved by crystallography for several spectrin tandem or multiple repeats (Davis et al., 2009; Grum et al., 1999; Ipsaro and Mondragon, 2010; Ipsaro et al., 2009, 2010; Kusunoki et al., 2004a,b; Stabach

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et al., 2009) and for the four repeats of α-actinin (Djinovic-Carugo et al., 1999; Ylanne et al., 2001). The remarkable feature of all these structures except the one of (Grum et al., 1999) is that they appeared as dimers.

Although α-actinin has only 4 repeats, which have all been solved by X-ray crystallography (Djinovic-Carugo et al., 1999; Ylanne et al., 2001), the other proteins have a high number of repeats, and no crystal for the complete protein have been successfully obtained for any of them. However, these structures could be crucial for the understanding of the interactions or the mutation modifications of these proteins. In that context, we previously proposed homology models for all the dystrophin repeats (Legrand et al., 2011). Herein, we have applied this approach to an exhaustive study of the repeats of human utrophin and human erythrocyte α- and β-spectrin. We compared them to the previously obtained models of dystrophin repeats and to the X-ray solved α-actinin repeat structure. We quantitatively assessed the similarity between these proteins at both the primary structure and the three-dimensional levels using electrostatic clustering methods and molecular hydrophobicity potential comparisons. We showed that spectrins and α-actinin on one hand and utrophin and dystrophin on the other hand shared some structural similarities, but a detailed molecular characterisation highlighted substantial differences. These differences are also related to a particular organisation of the exon and repeat coding in dystrophin and utrophin not observed in the other three proteins of the spectrin family.

## 2. Materials and methods

### 2.1. Sequence alignment and isoelectric point calculations

The sequences of human skeletal muscle utrophin and dystrophin and α- and β-spectrin from human erythrocytes were retrieved from the NCBI Protein Database, and sequences of α-actinin were retrieved from the deposited Protein Data Bank data (PDB: 1HCI) (see Fig. 1A for a schematic drawing of the proteins). To define the boundaries of the repeats, the alignment of dystrophin and utrophin repeats from Winder was used (Winder et al., 1995). The five proteins are schematically showed in Fig. 1A. Note that for α-spectrin, the repeat number according to Winder et al. (1995) was 20 complete repeats while in some papers, the SH domain accounts for a 10th "domain" of the protein which shifts the number to 21 "domains" (An et al., 2004; Li et al., 2010). We chose to number them from R1 to R20 according to Winder et al. (1995).

The sequences were aligned using the ClustalW2 program with the default parameters (Thompson et al., 1994; Larkin et al., 2007). The matrix similarity between the sequences was calculated using the Protdist program included in the PHYLIP package (Felsenstein, 1987). Isoelectric points were computed from the primary sequence using ProtParam on the ExPASy server (Gasteiger et al., 2005).

### 2.2. Homology modelling

The three-dimensional models were computed by the I-TASSER server (Zhang, 2008, 2009). The sequences of two successive tandem repeats were submitted with an overlap of one repeat for the next submission, i.e., first repeat 1–2, and then repeat 2–3. The two tandem repeats of utrophin that include known hinges, R3-4 and R17-18, were omitted. The incomplete repeats -1 (α) and 17 (β) of the spectrins were not modelled. This strategy was used to obtain models for the potential helical linkers between adjacent repeat pairs. I-TASSER produced one to five models for each of the two-repeat sequences submitted, and only the model with the best C-score for each tandem repeat was retained. We did not use the X-ray solved structures of the erythrocyte spectrins to compare all the models obtained by the same method. However, the templates used by I-TASSER are all the X-ray crystallography structures of the spectrin repeats. In all, 74 models were obtained: 21 for dystrophin, 19 for utrophin, 19 for α-spectrin and 15 for β-spectrin. For α-actinin, we used the X-ray crystallographic four-repeat structure available in the PDB (Djinovic-Carugo et al., 1999) and subdivided it into three structures for each tandem repeat.

### 2.3. Surface-property comparisons: hydrophobicity

Surface hydrophobicity calculations were performed using the PLATINUM program, which is designed to match hydrophobic patches for the prediction of receptor–ligand complexes (Efremov et al., 2007; Pyrkov et al., 2009). This program allows the calculation and visualisation of molecular hydrophobic/hydrophilic surface properties using the concept of molecular hydrophobicity potential (MHP). The PLATINUM algorithm also provides the hydrophobic and hydrophilic composition (in %) of the molecular surfaces following the MHP definition. All the models were submitted to the PLATINUM web site, which provided potential maps visualised using the PyMOL program.

### 2.4. Surface-property comparisons: electrostatics

The web-based version of the PIPSA program (Protein Interaction Property Similarity Analysis) (Blomberg et al., 1999; Gabdoulline et al., 2007; Richter et al., 2008; Wade et al., 2001) was used to compare tandem repeats with respect to their electrostatic potentials. PIPSA quantifies the similarity in the surface properties of homologous proteins, which is particularly useful for comparing the surface properties of the tandem repeats of the spectrin family members. After the 3D alignment fit, the models were submitted to the PIPSA server (Richter et al., 2008), and the Adaptive Poisson–Boltzmann Solver (APBS) software was used to calculate the electrostatic potentials (Baker et al., 2001). To prevent the potential discrepancies observed in calculations performed at too high or low an ion concentration, we chose to present maps computed for an ionic strength of 50 mM. The similarity indexes (SI) for each pair of surface electrostatic potentials were calculated (Blomberg et al., 1999) and converted into a distance matrix ranging from 0 to 2 (Gabdoulline et al., 2007; Wade et al., 2001). The distance matrix was then subjected to a clustering procedure, and the corresponding heat map was generated. A visualisation of the electrostatic potentials projected on the molecular surfaces of the repeats was produced using PyMOL.

## 3. Results

### 3.1. Intramolecular sequence similarity

The sequences were aligned using ClustalW2 (Thompson et al., 1994; Larkin et al., 2007) (Supplementary Fig. S1), and the similarity matrix between repeats was calculated using the Protdist program included in the PHYLIP package (Felsenstein, 1987). Only a poor sequence similarity was found between each single coiled-coil repeat of the five proteins. The pairwise sequence similarity scores of all repeats within each protein (Fig. 1B) indicated a median value below 0.2. The highest internal sequence similarity scores were 0.38 and 0.30 for the α-spectrin and β-spectrin repeats, respectively. It is also interesting to highlight that the highest similarity recorded between repeats for the two spectrin molecules was observed for repeats that are distant in the filament: R5 and R13 (0.38), R1 and R14 (0.35) and R2 and R10 (0.35) for α-spectrin and R4 and R15 (0.30) for β-spectrin. The best similarity value for

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