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Hypothesis

α-Actinin and spectrin structures: an unfolding family story

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Abstract In red blood cells, the integrity of the spectrin network is essential for normal cell shape and elasticity. To understand the molecular basis for spectrin's mechanical properties, one must determine how spectrin subunits interact with each other. The newly described crystallographic structures of two consecutive homologous repeats of human α-actinin, a member of the spectrin superfamily, shed new light on α-actinin interchain binding properties. Here I present evidence that interchain binding at the tail end of the spectrin molecule is likely to occur via a mechanism similar to that observed for α-actinin.

Key words: Spectrin; α-Actinin; Structure; Electrostatic interaction

1. Evolutionary relationship between α-actinin and spectrin

The relevance of α-actinin's structure to that of spectrin is based on the common evolutionary origin of both genes. Comparison of specific features in α-actinin and both spectrin subunits suggests the prior existence of a common α-actinin-like ancestor gene [1,2]. The analysis of spectrin repeat sequences supports a two-step model for the evolution of the spectrin superfamily [2]. Both steps of the model are outlined below.

The closest common ancestor of α-actinin and spectrin contained four homologous repeats. In step 1 (Fig. 1), a gene duplication gave rise to a stable lineage leading to modern α-actinin genes, while the other duplicated gene acquired additional repeats by a series of unequal crossing-over events. The genetic rearrangement produced the spectrin subunit ancestor, an elongated α-actinin-like protein. This spectrin ancestor formed an antiparallel homodimer capable of crosslinking actin filaments. The molecular basis of self-association at each end of this homodimer is similar to that of α-actinin self-association.

In step 2, one large gene was split into two functional genes, each encoding a different spectrin subunit (Fig. 1). This corresponded to a switch from a homodimer to a tetramer at the protein level. Each tetramer consisted of two heterodimers, each with an interchain binding at one end (tail end) identical to the site responsible for self-association of the long homodimer.

At the other end of the heterodimer (head end), modern interchain binding is due to the formation of a triple-helix bundle, which is the characteristic structure of a complete repeat. In the bundle, α-spectrin contributes one α-helix and β-spectrin two helices. This suggests that the gene cleavage responsible for the formation of the α- and β-spectrin genes occurred within a homologous repeat and directly resulted in a prototypic head-to-head association site between two heterodimers. An attractive feature of this event is that it would maintain the actin-crosslinking distance imposed prior to the cleavage of the ancestor gene.

2. α-Actinin self-association and spectrin tail-end interchain binding occur via similar mechanisms

In α-actinin, the four repeats (R1–R4) of the rod domain are involved in homodimer formation. Sequence analysis [1] shows that α-actinin repeats R1 and R2 are homologous to β-spectrin repeats β2 and β3 respectively, whereas repeats R3 and R4 are homologous to α-spectrin repeats α20 and α21 respectively (Fig. 2). Therefore, a conserved mode of interaction between α-actinin monomers and between spectrin subunits at the tail end of the tetramer is a likely consequence of the evolutionary relationship between α-actinin and spectrin subunits. This also suggests that the two first and last repeats of the long spectrin precursors were subject to selective constraints imposed by their role in antiparallel self-association. Furthermore, the α-actinin repeats and corresponding spectrin repeats are separated by unique and conserved linkers longer than the three residues usually found between other spectrin repeats.

The crystallographic structure of the two central repeats (R2 and R3) from the rod domain of human α-actinin in both monomeric and dimeric form [3] provides invaluable clues to the molecular basis of α-actinin self-association and spectrin tail-end interchain binding. In the homodimer, repeat R2 of one chain interacts with repeat R3 from the other chain and vice versa. Both repeats fold into similar triple-helix bundles and are linked by a continuous α-helix encompassing the third α-helix (3) of R2 and the first α-helix (1′) of R3 (Fig. 3).

Three types of interaction are observed: electrostatic interactions between negatively charged residues from R3 and positively charged residues from R2; hydrophobic interactions between the loop connecting α-helices in R3 and residues from R2; Van der Waals interactions and a hydrogen bond network between small residues from R2 and R3. The charge distribution on the surface of the triple-helix structure is a striking feature of the R2 and R3 repeats: R2 (as well as R1) is positively charged and R3 (as well as R4) is negatively charged. Therefore, the rod domain of α-actinin has an electrostatic polarity that defines the register between repeats in the antiparallel homodimer.

The mechanism of spectrin tail-end interchain binding may

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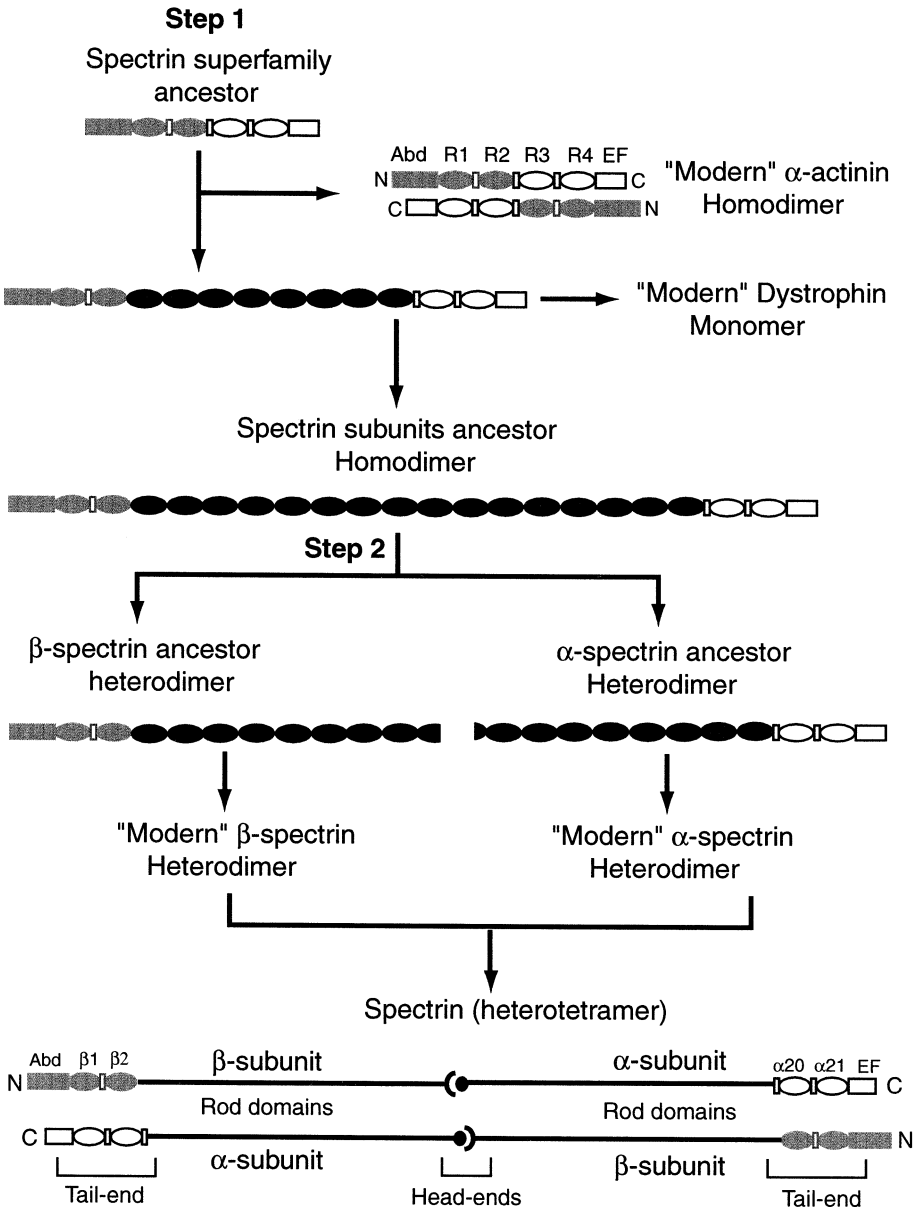

Fig. 1. Model of the evolution of the spectrin superfamily. This diagram is derived from Thomas et al. [2]. Step 1: Gene duplication and gene rearrangements gave rise to the various members of the spectrin superfamily. The successive addition of repeat sequences resulted in the formation of the spectrin subunit ancestor, an elongated α-actinin-like protein. This step resulted in an early length determination of each spectrin superfamily member predating the divergence of arthropods and vertebrates. Step 2: Genes encoding each spectrin subunit probably arose from the cleavage of the gene encoding the elongated α-actinin-like ancestor. Consequently, the repeats responsible for the spectrin subunit ancestor or α-actinin self-association were split between two distinct polypeptide chains still capable of forming a heterodimer and now able to form a heterotetramer. Symbols: repeats (ovals), octameric linkers (narrow rectangles). The non-repetitive segments Abd (actin binding domain) and EF (calmodulin-like domain) are represented by elongated rectangles. Spectrin and α-actinin repeats involved in interchain binding are indicated.

be similar to that of α-actinin. This hypothesis is supported by three observations

(1) With a few exceptions, the residues in R2 and R3 involved in dimerization are conserved in spectrin repeats β3 and α20 (Fig. 2). Hydrophobic residues contained in the loop linking helices 1′ and 2′ of R3 are not conserved in α20. Nevertheless, a hydrophobic interaction similar to that between the 1′ and 2′ loops and the leucine residue found at the N-terminal portion of R2 helix 1 is likely to occur in spectrin where the equivalent leucine residue is conserved. The length of the loop linking the second and third helices of α-actinin R3 versus spectrin α20 also differs by seven residues. Despite the difference in length, three out of four residues from the α-actinin loop involved in self-association are conserved in all α20 repeats (Fig. 2).

(2) Among the conserved residues, the abundance of acidic residues at the C-terminal end of the putative first α-helix in spectrin's α20 is noteworthy (Fig. 2). Based on sequence alignment, two of these acidic residues (labeled with a star in Fig. 2) are not only conserved in α20 repeats across species but acidic residues are found at the corresponding positions in less than 2% of 152 other α or β spectrin repeats of known se-

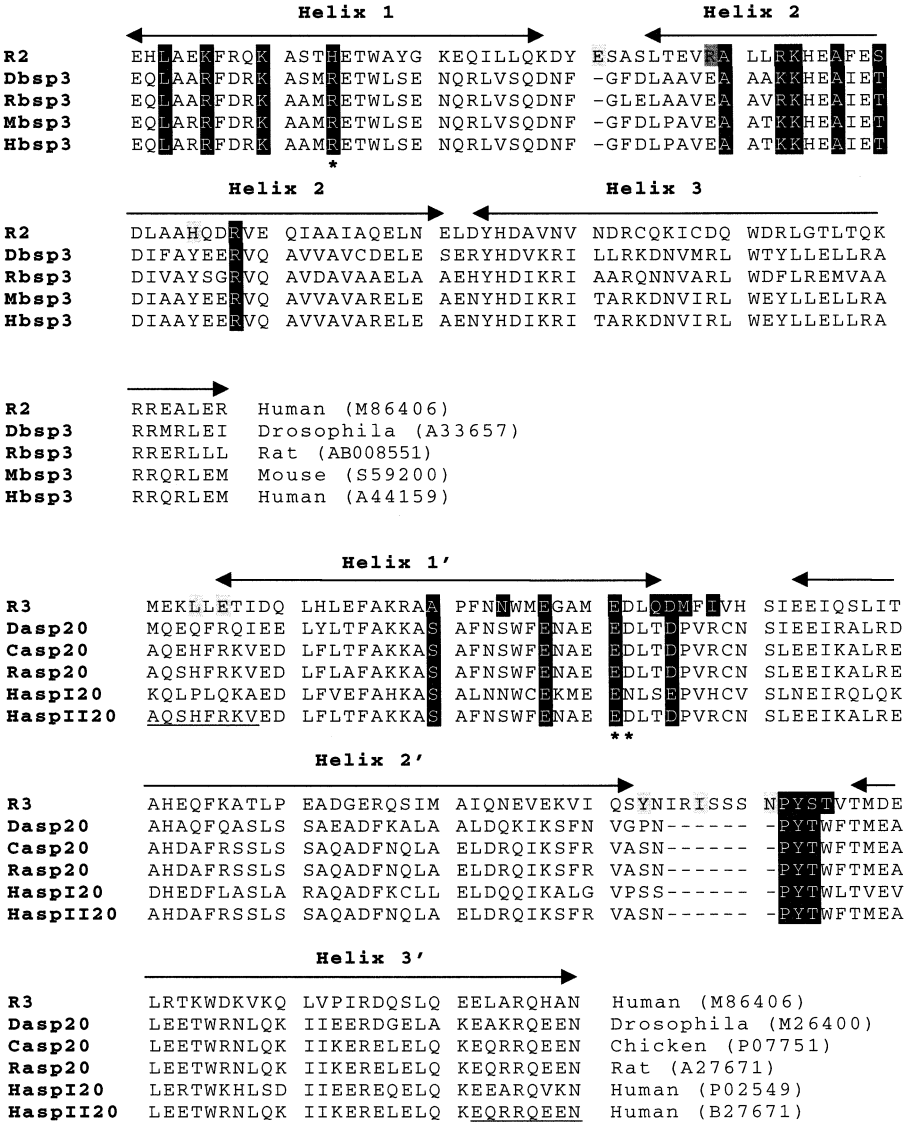

Fig. 2. Sequence alignments of α-actinin and spectrin homologous repeats. Repeats R2 and R3 from human α-actinin (muscle isoform) were used as a reference in the sequence alignment containing spectrin repeats β3 and α20. α-Actinin residues involved in dimer formation and their homologs in spectrin repeats are highlighted in black. Long linkers between repeats are underlined. α-Actinin residues involved in direct and indirect inter-segment interactions and not conserved in spectrin repeats are highlighted in gray. Acidic and basic residues unique to repeats involved in spectrin tail-end interchain binding are indicated with a star.

quence (data not shown). Similarly, basic residues involved in the electrostatic interactions between α-actinin's R2 and R3 repeats are also conserved in spectrin's β3 repeat (Fig. 2). A basic residue (labeled with a star in Fig. 2) conserved in the putative first α-helix of β3 repeats has no equivalent in any other known α- and β-spectrin repeat (data not shown). For the other conserved basic residues, the frequency found at the corresponding positions in the first α-helix of other α- and β-spectrin repeats varies from 26% to 8.5%. The charges at the surface of spectrin repeats involved in tail-end binding suggest that the electrostatic polarity observed in α-actinin is conserved in spectrin, where the first two repeats of β-spectrin are positively charged and the last two repeats of α-spectrin are negatively charged. Therefore, the antiparallel subunit association in spectrin dimers is probably dictated by electrostatic interactions, confirming the hypothesis that during spectrin assembly the first interaction between subunits occurs at the tail end of the molecule [5].

(3) The electrostatic interactions between R2 and R3 result in a specific lateral register of these repeats. Therefore, if the spectrin tail-end interaction is similar to that in the α-actinin dimer, the lateral register of spectrin repeats should be critical for spectrin assembly. Indeed, in vitro binding experiments show that the deletion the β2/β3 or α20/α21 octameric linkers prevents interchain binding whereas four different substitutions of conserved residues in the β2/β3 octamer have no effect on interchain binding [6,7]. These data demonstrate the role of the octamers in defining the relative register between repeats at the tail end of spectrin. As expected from the α-actinin crystallographic data, residues from these linkers do not form distinct interchain binding sites and are likely to adopt an α-helical structure. The α-helical continuity between the last α-helix of a segment and the first α-helix of the next segment is not limited to repeats involved in interchain binding. Indeed, the α-helical continuity between adjacent repeats and their side-chain interactions hypothesized by Yan et al. [8]

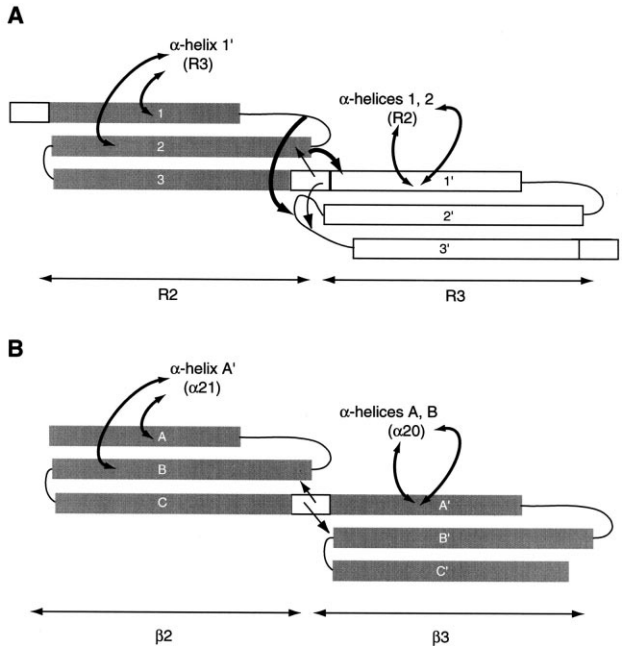

Fig. 3. Diagrammatic representation of contiguous repeats. A: The 55° rotation between α-actinin repeats R2 and R3 allows for structural complementarity between the two subunits in the homodimer. Double-headed arrows identify the α-helices from R2 and R3 involved in dimer formation. The thin arrows represent the interaction between the R2/R3 linker and the flanking repeats. The thick arrows represent the direct inter-segment interactions. B: Conserved sequences between R2 and R3 α-actinin repeats and β3 and α20 spectrin repeats suggest that at the tail end of spectrin, segments β2 and β3, faces segments α21 and α20 respectively. The hypothesized interactions between repeats are indicated by double-headed arrows. Thin arrows represent the β2/β3 octamer interactions with flanking repeats. Note that direct inter-segment interactions similar to that found in α-actinin are not possible (see text for details).

have been confirmed by the crystallographic structure of two contiguous α-spectrin repeats [9].

3. Repeat stability

In addition to defining a lateral register of repeats, the linkers are involved in the stabilization of flanking repeats. Crystallographic data [3] show hydrophobic interactions between conserved residues from the linker separating α-actinin repeats R2 and R3, and residues from helix 2 in R2 and the 2′–3′ loop in R3 (Fig. 3, thin arrows). Repeat stability is further enhanced by direct inter-segment interactions between loops 1 and 2 in R2 and loops 2′ and 3′ in R3, and between the N-terminal residues of helix 2 and helix 3′ (Fig. 3, thick arrows). Interestingly most of these residues are not conserved in spectrin repeats β2 and α20 (Fig. 2, residues labeled in gray), raising questions about the nature of inter-segment stabilization. It is not surprising that α-actinin residues involved in the stabilization of R2 and R3 are not conserved in spectrin, since the corresponding repeats (β3 and α20 respectively) are not found in the same spectrin subunits and thus are not subjected to the same selective pressure as R2 and R3. Nevertheless, inter-segment stabilization was demonstrated for *Drosophila* spectrin where an amino acid substitution (Arg to Pro) within the β2/β3 octamer does not affect interchain binding but substantially increases proteolytic sensitivity of the mutant β-spectrin polypeptide [7]. Therefore, spectrin inter-segment stability probably relies on side-chain interactions with conserved residues in the octamers since none of the α-actinin residues responsible for direct inter-segment interaction are conserved in spectrin α20 and β3 (Fig. 3). The nature of inter-segment stabilization between spectrin repeats β2 and β3 or α20 and α21 is probably similar to that existing between segments R1 and R2 or R3 and R4 respectively.

4. Conclusion

I propose the following model for spectrin tail-end interchain binding. Electrostatic interactions between negatively charged residues displayed at the surface of repeats α20 and α21, and positively charged residues displayed at the surface of repeats β2 and β3, are responsible for the antiparallel association of spectrin subunits. The α21 and α20 repeats are aligned with β2 and β3 respectively, and the octamers found between these repeats define the relative register between α- and β-spectrin repeats. Additional interactions between the non-repetitive β1 and α22 repeats [8] as well as weak interactions between other repeats within the rod domain contribute to the overall stability of spectrin's tail ends. Tail-end interchain binding is a critical initial event in the assembly of the spectrin network. Indeed, in heterozygous individuals carrying the αLELY gene, the corresponding subunit with defects in interchain binding is not found at the plasma membrane [4,5].

Based on sequence comparisons, the characteristic features of the repeats and linkers necessary for subunit interactions were probably present in the common ancestor of α-actinin and spectrin, and were conserved in both lineages. In contrast, the human dystrophin sequence lacks these characteristic features including the elongated linkers. This suggests that dystrophin is either monomeric [10] or self-associates by a different mechanism.

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