

Mini review

SPECTRIN AND PHOSPHOLIPIDS – THE CURRENT PICTURE OF THEIR FASCINATING INTERPLAY

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Abstract: The spectrin-based membrane skeleton is crucial for the mechanical stability and resilience of erythrocytes. It mainly contributes to membrane integrity, protein organization and trafficking. Two transmembrane protein macro-complexes that are linked together by spectrin tetramers play a crucial role in attaching the membrane skeleton to the cell membrane, but they are not exclusive. Considerable experimental data have shown that direct interactions between spectrin and membrane lipids are important for cell membrane cohesion. Spectrin is a multidomain, multifunctional protein with several distinctive structural regions, including lipid-binding sites within CH tandem domains, a PH domain, and triple helical segments, which are excellent examples of ligand specificity hidden in a regular repetitive structure, as recently shown for the ankyrin-sensitive lipid-binding domain of beta spectrin. In this review, we summarize the state of knowledge about interactions between spectrin and membrane lipids.

Keywords: Spectrin–phospholipid interactions, Spectrin repeats, Spectrin tetramers, Ankyrin, Erythrocytes, Actin-binding domain, Pleckstrin homology domain, Dystrophin, Lipid bilayer, Membrane skeleton

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Abbreviations used: ABD – actin-binding domain; AE1 – anion exchanger 1; Ank – ankyrin; CH – calponin homology domain; GPC – glycophorin C; MPP1 – membrane palmitoylated protein 1; PC – phosphatidylcholine; PE – phosphatidylethanolamine; PH – pleckstrin homology domain; PI – phosphatidylinositol; PI(4,5)P₂ – phosphatidylinositol-4,5-bisphosphate; PS – phosphatidylserine; SH3 – SRC homology 3 domain; UPA – Unc5-PIIDD-ankyrin domain; ZU5 – domain present in ZO-1 and Unc5-like netrin receptors

INTRODUCTION

The biconcave disk shape and deformability of erythrocytes are related to the mechanical properties of their composite plasma membrane. It consists of a lipid bilayer and a membrane skeleton network. Spectrin, first described in erythrocytes by Marchesi and Steers, is the main component of the latter [1]. Two spectrin heterodimers, composed of laterally interacting α - and β -subunits (280 and 247 kDa in size, respectively) [2–4], interact with each other in a head-to-head manner to form antiparallel heterotetrameric filamentous units [5, 6]. Three to six spectrin tetramers bind at either end of the tetramer to one regular 37-nm long rod actin protofilament, the latter forming the horizontal axis of a structure known as the junctional complex. Recent data revealed an average spectrin tetramer length of 46 nm, which is much less than the length of the fully extended molecule (approximately 190 nm) [7–11]. The prevalence of higher spectrin oligomers (hexamers and octamers) in almost every junctional complex of the network has also been observed [10, 12]. The elastic two-dimensional spectrin-based network, attached to the cytosolic side of the erythrocyte plasma membrane, is associated with a number of membrane integral proteins (Fig. 1) [13]. In mammals, seven genes code for spectrin subunits: two for α -spectrins (α I- and α II-spectrins), four for conventional β -spectrins (β I- to β IV-spectrins), and one for an ortholog of the β H (heavy) subunit (β V) [14]. The most common heterodimeric α I/ β I-spectrin is expressed in the erythrocytes, and α II/ β II spectrin is expressed in most if not all vertebrate cells.

The diversity of the products of the seven genes can be correlated with the structural complexity of cells. These multiple protein isoforms are distributed to various intracellular locations besides the plasma membrane, including the Golgi cisternae and vesicles, the endoplasmic reticulum, and the nucleus [13, 15].

One characteristic structural feature of the spectrin family proteins [5] is the spectrin repeat, which is an approximately 106-amino acid residue long, α -helical repeat motif that forms a triple helical coiled coil (Fig. 1) [16]. α -spectrin has 20 full-length repeating units and one incomplete motif of single α -helix (at N-terminus), while β -spectrin has 16 complete repeats and one partial segment consisting of two helices. Both incomplete segments interact with each other to restore the triple helical repeat structure while forming the spectrin tetramer [17]. According to the spectrin flexibility model, the repeats may act as a molecular spring and respond to the shearing forces with at least partial unfolding of the coiled coil.

In addition, between the 9th and 10th segments, α -spectrins also contain a 60-residue long SH3 domain [18], which is a common structural motif found in proteins engaged in signal transduction, proliferation and cellular motility [19]. Its function is mostly executed by binding to proline-rich segments of protein ligands with PXXP as a minimum consensus sequence [19, 20]. α II-spectrin differs from α I-spectrin by a 36-residue insert within the α 10 repeat (named the CCC-loop, for calmodulin, calpain, caspase) [21]. This bears a Ca^{2+} -calmodulin-

binding site and cleavage sites for both caspases (2, 3 and 7) and for m- and μ -calpain [22–24]. Calpain and caspase cleavage is regulated by Ca^{2+} -calmodulin [25] and by phosphorylation of Y1176 residue, which is located in the calpain recognition site [26, 27]. However, the physiological importance of this site remains unknown.

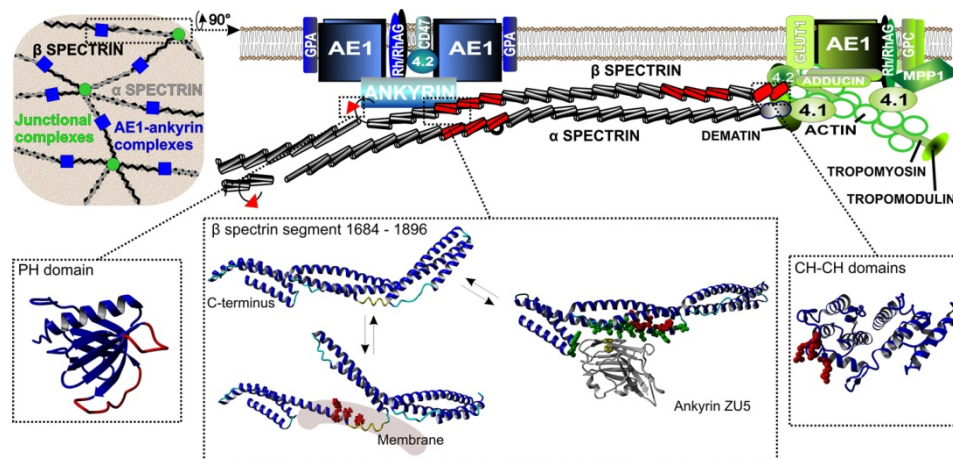


Fig. 1. The spectrin skeleton of the erythrocytes. Upper part – Spectrin dimers (α -subunit gray strands, β -subunit black strands) associate to form predominantly tetramers (but also hexamers and octamers) and bridge junctional complexes (green). The latter, together with AE1-ankyrin complexes (blue) are major membrane skeleton macrocomplexes that anchor the spectrin network to the plasma membrane. The membrane-binding domains of spectrin are marked red. For further details see the main text and [13]. Lower part – Major structural motifs of spectrin engaged in phospholipid bilayer recognition. PH domain – PDB ID: 1WJM (loops important for $\text{PI}(4,5)\text{P}_2$ binding in red). The triple helical repeats are represented by the ankyrin-sensitive lipid-binding domain within the 14th segment of β -spectrin (ribbon representation of segments 13–15). Membrane binding results in the “open” conformation (amino-acid residues essential for lipid binding in red: W1771, L1775, M1778, W1779, all within helix 14C; 3_{10} helix is yellow). Alternatively, steric interference upon the interactions of spectrin with the ZU5 domain of ankyrin (gray) blocks the “opening” of the triple helical bundle – PDB ID: 3KBT (amino-acid residues crucial for ankyrin binding in green, residue F917 of ZU5 in yellow). Tandem CH domains of α -actinin in its “closed” conformation – PDB ID: 1WKU (putative $\text{PI}(4,5)\text{P}_2$ -binding residues in red). Other domains of spectrin: SH3 (black arc) and EF-hands (gray merged circles).

The C-terminal domain of α -spectrins consists of two EF-hand motifs (calcium-dependent and calcium-independent) related to calmodulin [28–31]. The function of these Ca^{2+} -binding motifs remains unknown.

On the other hand, β -spectrins contain two CH (calponin homology) domains in tandem configuration at their N-terminal region. This forms the actin-binding domain. This domain is also found in many other actin-binding proteins of different functions, ranging from actin cross-linking to signaling (Fig. 1) [32, 33].

β -spectrins in their “long” isoforms [13] have a region of homology named the pleckstrin homology (or PH) domain at their C-terminus. It is similar to motifs found in many proteins involved in cellular signaling and cytoskeletal organization (Fig. 1) [34–36].

The principal anchorage of spectrin to the membrane in erythrocytes is mediated by the interaction of the β -subunit with ankyrin (Fig. 1) [37–40]. The conserved region within repeats 14 and 15 of β I-spectrin binds erythroid ankyrin with high affinity [41–43]. The early studies of Lu et al. indicated that ankyrin exhibits different affinities for spectrin dimers ($K_d = 2.5 \pm 0.9 \mu\text{M}$) and tetramers ($K_d = 0.27 \pm 0.08 \mu\text{M}$). Additionally, the phosphorylation of ankyrin decreases its affinity for either phosphorylated or unphosphorylated spectrin tetramers ($K_d = 1.2 \pm 0.1 \mu\text{M}$), but not spectrin dimers [44]. Isolated domains responsible for spectrin–ankyrin contacts interact with each other with a K_d of $0.19 \pm 0.03 \mu\text{M}$ [42].

The major isoform of erythroid ankyrin is composed of three distinct regions: an N-terminal domain known as a membrane domain containing 24 ankyrin repeats, a central region known as a spectrin-binding domain containing three subdomains (ZU5A, ZU5B and UPA), and a C-terminal regulatory domain with its subdomain called the “death domain” (DD). Only the first of the two ZU5 domains (ZU5A) of erythroid ankyrin is directly responsible for binding β -spectrin [43, 45–47]. Furthermore, the ZU5A-ZU5B-UPA-DD tandem of erythroid ankyrin seems to be crucial for auto-regulation of ankyrin functions [46]. Going further, the membrane-binding domain of erythroid ankyrin interacts with dimers of anion exchanger 1 (AE1) via the cdb3 domains of the latter [48]. A recently published model indicates that an extensive peripheral area of the cdb3s of the first dimer binds ankyrin repeats 18–20 primarily through hydrophobic interactions [49]. Furthermore, the cdb3s of the second dimer bind ankyrin repeats 7–12 [50]. The spectrin–ankyrin–AE1 complex is stabilized by protein 4.2, together with several other proteins specific to erythrocytes, such as the proteins of the Rh complex (Rh and RhAG; Fig. 1). Major components of the latter in erythrocytes are Rh polypeptides, CD47, and the LW blood group antigen glycoprotein [51, 52]. AE1 interacts with other integral membrane proteins such as glycophorin A (GPA) and glycophorin B (GPB).

In the cell membrane, there is a second skeleton–membrane link, the macro-complex of membrane proteins coordinated by actin protofilament. This junctional complex consists of the transmembrane proteins (Fig. 1): glycophorin C (GPC), Rh complex proteins, the anion exchanger 1, and glucose transporter-1 (GLUT1), and the chemokine receptor Duffy (DARC) and the Kell/KX transporter complex. In addition, the constitutive components of the junctional complex are also peripheral proteins: actin, protein 4.1, p55/MPP1, adducin, tropomyosin, tropomodulin, dematin, and protein 4.2. All of these proteins play essential roles by increasing spectrin–actin affinity or regulating protein interactions in the spectrin–actin junctions [53–56]. The experimental data indicate the crucial role of protein 4.1 in the organization of this complex, which is mostly due to the formation of the core ternary complex of proteins

4.1R–MPP1–GPC [56, 57]. The published data revealed that the N-terminal α -helix-rich region of protein 4.1R binds to the cytoplasmic tail of transmembrane proteins, including GPC, and the C-terminal lobes of the FERM domain of protein 4.1R interact with the membrane-associated protein MPP1 (p55) [58, 59]. Furthermore, both adducin and dematin form a complex with glucose transporter-1 and the ends of the spectrin tetramers. Most recently, it appeared that adducin also interacts with AE1, and this interaction is essential for maintaining the normal, biconcave disk shape and resilience of erythrocytes [53, 55, 60]. Another important binding site stabilizing the spectrin–actin junctional complex is mediated via protein 4.2, which binds to AE1 dimers and the EF-domain of erythroid α -spectrin (i.e. near a site which is spatially close to the 4.1 binding site in the spectrin tetramer) [61].

In recent years, increasing evidence shows an important role of the interactions between spectrin and membrane lipids, not only for cell membrane integration under physiological conditions, but also for the potential recovery of membrane integrity in certain pathological states, particularly in hereditary spherocytosis (HS) and hereditary elliptocytosis (HE) [62–65]. This paper focuses on recent updates on the interactions between spectrin and phospholipids.

Spectrin binds the phospholipid bilayer

Several decades of studies on model systems and erythroid and non-erythroid cells show that direct spectrin–phospholipid interactions contribute to the anchorage of the membrane skeleton to the biological membranes. The earliest published studies on the interaction of spectrin with phospholipids were carried out on extracts mainly consisting of a spectrin–actin mixture [66–68]. In the following decade, hydrophobic and amphipathic ligands were discovered to interact with purified spectrin, which confirmed the presence of potentially hydrophobic sites in the spectrin structure [68–74]. These and later studies have been summarized in previous reviews [75, 76].

Early studies of spectrin–lipid interaction, dating back to the nineteen eighties, revealed a larger effect exerted by the protein on the fluidity of mono- and bilayers composed of anionic phospholipids when compared to zwitterionic counterparts [66]. However, the majority of subsequent studies showed that the affinity of purified spectrin is similar for both PS-containing and pure PC vesicles [71, 77–79]. Using a pelleting assay approach, Diakowski and Sikorski were first to show the lipid-binding activity of non-erythroid spectrin [80]. Remarkably, no reasonable differences for liposomes prepared from either PC or PC mixtures with PE or PS were reported. There was also an indication that the interaction of spectrin with the PS-containing monolayer was Ca^{2+} dependent [66, 81], but this possibility was never explored further. It is possible that complexation of Ca^{2+} would affect PS-binding as is known for annexin-type proteins [82, 83], or have an effect on spectrin conformation and its sensitivity to calpains [25, 84, 85].

The finding that erythroid spectrin binds PE-rich mono- and bilayers [77, 79, 86] was very important. PE, similarly to PS, is asymmetrically distributed within the plasma membrane and located almost exclusively in the cytosolic leaflet of the plasma membrane [87]. Moreover, the inner monolayer of the erythrocyte membrane contains more PE than PS [87, 88]. Such specificity was also seen in the case of non-erythroid spectrin, as reported by Diakowski et al. [89], who showed that purified brain spectrin induced an increase in surface pressure in lipid monolayers composed of PE/PC, PS/PC and PC. The strongest effect of spectrin was observed on monolayers containing 50–60% of PE. Interestingly, purified brain spectrin also bound the PI or PI/PC more efficiently than erythroid spectrin [90]. Subsequent studies showed that isolated, NaOH- and protease-treated natural erythroid membranes bind spectrin with affinities similar to those obtained for model phospholipid membranes, indicating the presence of spectrin receptors that are NaOH- and protease-resistant [91].

It is well known that the fluidity and lipid packing of the membrane stem from the intrinsic properties of its constituents. Primarily, the degree of acyl chain saturation of phospholipids and the cholesterol content strongly modulate the properties of lipid membranes. However, the interplay between individual lipid species is rather complex, which may result in lateral segregation and membrane sub-compartmentalization [92, 93]. This is also behind the fact that the effect of membrane lipid fluidity on spectrin–phospholipid interactions is not simple, but depends on both cholesterol content and the chemical structure of the acyl chains of the membrane lipids. Experimental results showed that a cholesterol content in the range of 10–20% in the egg PE/PC monolayer facilitates its penetration by erythroid and non-erythroid spectrins, as does an increase in phospholipid chain length. Additionally, it was also observed that the spectrin-binding capacity of liposomes prepared from a raft-like [92, 94] mixture of lipids increases compared to that of standard liposomes prepared from the egg PE/PC mixture. Subsequent studies revealed that direct spectrin–lipid interactions might affect the lateral lipid organization [95]. These results demonstrate that spectrin could participate in sustaining the two-dimensional order in the lipid domains through direct interaction with the head groups of aminophospholipid species.

Moreover, the analysis carried out by Manno et al. [96] revealed that the phospholipid distribution modulates erythrocyte membrane elasticity through altered spectrin–phospholipid interactions. The mechanism is based on ATP-dependent glycation of erythrocyte membrane-associated spectrin after disruption of lipid asymmetry [97]. Spectrin associated with membranes could be glycated only after ATP depletion and consequent translocation of phosphatidylserine (PS) from the inner to the outer lipid monolayer. It has been shown that the interaction of the lysine and arginine residues of erythrocyte spectrin with PS makes those residues inaccessible for glycation by glucose and ribose. Those glycation processes involve repeats 3, 4, 12, 13 and 14 of β -spectrin and repeats 8, 9 and 10 of α -spectrin. These observations may suggest that erythrocytes, which are continuously exposed to glucose in the plasma

during their circulatory life span, have an ATP-dependent protective mechanism against spectrin glycation.

Phospholipid-binding sites in erythroid spectrin

Spectrin is a multidomain and multifunctional protein with specific structural regions, including several putative lipid-binding sites (Fig. 1). It is possible to distinguish different types of lipid-binding sites in the erythroid and non-erythroid spectrin structure. The ability to bind phospholipids is a characteristic feature of the spectrin repeat motif [98–100]. What appeared to be significant for the physiology of the erythrocytes is the fact that β -spectrin has a relatively high affinity towards specific aminophospholipid-rich (PS and/or PE) membrane compartments [101–104].

The other important issue was the precise localization of the lipid-binding site(s) within the spectrin molecule. It took quite a bit of time to determine that the PE-binding site is located at one of the terminal domains of dimeric spectrin [86]. Further studies were carried out by An et al. [101, 102] with fragments of erythroid spectrin covering the whole molecule used for testing their binding affinities to liposomes prepared from PS and PS/PC. The obtained data indicate that the PS-binding sites are located in repeats 8–10 of α -spectrin, and in repeats 2–4 and 12–14 and at the N-terminal region of β -spectrin (Fig. 1). It can be concluded that PS-binding sites in the beta-spectrin are localized near the sites of attachment of either erythroid ankyrin or protein 4.1, both bridging spectrin with the transmembrane complexes. These data may support the hypothesis that the interaction of spectrin with PS may modulate protein–protein interactions in the erythroid membrane. Subsequently, other studies showed that the PE-binding site is located within the 38-residue N-terminal fragment of the erythroid β -spectrin ankyrin-binding domain (residues 1768–1805; Fig. 1) [103]. A functionally similar site was found at the corresponding stretch of the sequence of non-erythroid spectrin (residues 1776–1906) [105].

The spectrin repeat is one of the three main structural components of proteins belonging to the spectrin protein family, which includes spectrin, dystrophin, utrophin, α -actinin, and spectraplakins (trabeculin/kakapo). The repeat domains of spectrin are responsible for a variety of functions, such as dimerization, recruitment of signaling molecules, interaction with cyto- or membrane skeletal elements, and indirect anchoring of these structures to the membrane lipid bilayer [13, 106, 107]. The majority of the information concerning the lipid-binding activity of the spectrin repeat motif is based on studies of human dystrophin. The central rod domain of this protein is composed of 24 spectrin repeats interrupted by four hinges that delineate three sub-domains. Early studies on dystrophin–membrane interactions were carried out using a series of constructs containing the second repeat of the rod domain [108]. DeWolf et al. showed that the native structure of that fragment could influence the properties of lipid membranes containing anionic phosphatidylserine [100]. The studies carried out by LeRumeur et al. have deepened the understanding of dystrophin–

lipid interactions [107]. Their studies demonstrated that the three first repeats (1–3), unlike the last five (repeats 20–24), strongly interact with anionic lipids. Subsequent studies showed that the major sub-domain (repeats 4 to 19) also interacts with membrane lipids [109], which appeared to be also true for the so-called actin-binding domain 2 (ABD2, as distinct from ABD1 which consists of two N-terminal CH domains) of dystrophin, in particular repeats 12 and 13 [98]. Additionally, it has been shown that this domain plays a crucial role in membrane mechanical resistance through interactions with lipid bilayers of different curvatures induced by the contraction–relaxation cycles of active muscles. According to the same authors [98, 107] the helical structure of the dystrophin rod domain is maintained, and the protein organization is modulated by the lateral pressure of the associated lipid monolayer. Moreover, recent data showed that the central domain of dystrophin contributes significantly to the stiffness and the stability of the sarcolemma through its simultaneous interactions with the cytoskeleton and lipid membrane [110]. The analysis carried out by Vie et al. [111] revealed that the interaction of the first sub-domain with membrane lipids does not lead to any changes in the secondary structure of the protein. On the other hand, interaction of dystrophin triple-helical repeats with membranes results in a reduction of the inter-helical interactions, suggesting the appearance of partly opened coiled-coil structures [109]. These conclusions concur with results obtained for repeats 14–15 of β -spectrin interacting with lipids [104, 112]. Together with a possible common mechanism for binding the membrane lipids by spectrin repeats (dependent on other membrane proteins) [104], this will be described later in this review. On the other hand, erythroid and neuronal spectrin, which both strongly bind membrane lipids, contain evolutionarily conserved lipid-binding triple-helical repeats, which show a much higher degree of sequence identity than corresponding repeats that do not share this property [113].

The second specific class of direct phospholipid-binding site is located in the N-terminal region of erythrocytic β -spectrin consisting of CH1–CH2 domain (ABD, actin-binding domain; Fig. 1). It has been observed that not only actin but also protein 4.1R binds to two calponin homology domains (residues 1–301) and the latter interaction is greatly enhanced by phosphatidylinositol-4,5-bisphosphate (PI(4,5)P₂), implying the existence of a regulatory switch in the cell [114]. Truncation of the CH2 domain (deletion of the first 20 amino acid residues) promotes binding of protein 4.1R and disrupts an actin-binding activity of CH1–CH2 domain. It was concluded that in the absence of PI(4,5)P₂, the N-terminal domain of spectrin β I adopts the “closed” conformation, which can be opened upon binding of PI(4,5)P₂. A similar model of actin-binding domain, which is formed by two consecutive calponin homology domains arranged in a “closed” conformation, was proposed for human titin and α -actinin (Fig. 1) [115, 116]. Two distinct crystal structures were solved and the conformation is controlled by a phospholipid-regulated intramolecular pseudoligand mechanism. There probably exists a distinct binding site for PI(4,5)P₂, where the fatty acid

moiety would be oriented in a direction that allows it to interact with the linker sequence between the second CH domain and the first spectrin repeat. In the case of α -actinin, the conformational changes of the actin-binding domain correlated with a structural flexibility between the tandem CH domains make actin-binding sites fully available for binding other ligands, and the phospholipid seems to be required for full activation of α -actinin.

PH-domain

Another spectrin molecule structural motif that recognizes phosphatidylinositol lipids is the pleckstrin homology (PH) domain, a characteristic extension found in the “long” isoforms of beta subunit. The 100- to 120-residue long C-terminal domain consists of two perpendicular anti-parallel beta sheets followed by a single helix (Fig. 1) [34, 117]. Intriguingly, similar domains are known to occur in numerous eukaryotic proteins involved in signal transduction, cytoskeletal organization [118] and membrane binding [119], but little is known about the physiological role of the PH domain of spectrin. The lipid-binding surface within the PH domain of beta spectrin is formed by positively charged residues, so electrostatic interactions with phosphate groups of the ligand seem to play a key role [117]. Although this domain possess relatively weak specificity and moderate affinity towards PI(4,5)P₂ [120], it has been proposed that its interaction with the membrane is a prerequisite for Golgi membrane homeostasis and vesicular transport along the axons [121, 122]. Most recently, it was found that beta spectrin with its PH domain is required for polarized membrane skeleton assembly in midgut copper cells of *Drosophila melanogaster* through a mechanism that is not related to PIP₂-binding activity [123].

Relationship between ankyrin-binding and lipid-binding activities

Early work by Sikorski et al. pointed to a possible functional relationship between two binding activities of erythroid spectrin towards phospholipids and ankyrin [74]. Subsequent research revealed that purified ankyrin inhibits erythroid spectrin–phospholipid interaction (60% inhibition for vesicles contained PE/PC 3:2, but only 10–20% for PS/PC) [77, 124]. Analogous results were obtained for tetrameric spectrin on lipid monolayers [125]. However, ankyrin inhibited only one of the two possible lipid-binding sites. A similar level of ankyrin-mediated inhibition of lipid interactions was observed for non-erythroid (brain) spectrin [89]. Moreover, it appeared that β -spectrin was inhibited much more strongly than α -spectrin [89, 125].

The ankyrin-binding domain of erythroid spectrin, first described by Kennedy et al. in 1991 [126], was found within the region-spanning 14th and 15th repeats of erythroid β -spectrin (Fig. 1). The ankyrin-binding site includes amino acid residues located in helices 14A and 14C as proposed by Ipsaro et al. [41], and side chains within a large helical linker between the two repeats and residues in the B-C loop of repeat 15, as suggested by Stabach et al. [127]. Further studies by La-Borde et al. confirmed the data [42], indicating that spectrin adopts a unique conformation during binding to ankyrin.

Mutational analysis of the spectrin-binding domain of erythroid ankyrin (ZU5A domain) showed that F917S substitution completely abolished the formation of the spectrin–ankyrin complex [45]. It has been suggested that this residue is crucial for the appropriate orientation of the spectrin-binding site in erythroid ankyrin. On the other hand, the minimal ankyrin-binding site in spectrin is supposed to be formed by negatively charged residues within helix 14C together with side chains in the loop between helices 15B/C. Direct interactions between erythroid ankyrin and spectrin are also mediated by several hydrophobic residues (in ankyrin: L914, V915, M918, T950; in spectrin: A1780, L1785, T1788, L1792). Interestingly, although F917Ank does not make a direct contact with spectrin, it is localized in a dense hydrophobic core (916SFMV919) and interacts with F913Ank, which is crucial for spectrin binding (Fig. 1) [45].

Studies carried out by Czogalla et al. [112, 128] based on the spin-label mobility and spin-spin distance measurements via electron paramagnetic resonance spectroscopy of the ankyrin-binding domain of erythroid β -spectrin preceded the reports on the atomic structure of this region by other three groups [42, 47, 129–131]. Structural information for the protein in the aqueous environment indicated that the examined lipid-binding site is a mixed $3_{10}/\alpha$ -helix, the amphipathic character of which is correlated with the unique conformation of its N-terminus [128]. These observations indicated the presence of exposable hydrophobic surfaces within the ankyrin-binding domain structure responsible for direct interactions with membrane lipids. It seemed straightforward that the 14th repeat of the erythroid β -subunit has a typical triple-helical structure. However, interaction with phospholipids (PE/PC liposomes) or detergents caused significant deviations in the tertiary structure of the analyzed fragment. The lateral interactions with phospholipid bilayers induced changes in the mutual orientation of the helices, which was reflected by the increasing distance between the spin labels on both of the helices [112]. Further studies by Paździor et al. confirmed these observations [132].

In recent studies, Wolny et al. [104] identified the amino acid residues responsible for lipid-binding activity within the ankyrin-binding domain of erythroid β -spectrin. The construction of several mutants of this domain bear different combinations of substitutions within the lipid-binding site and these are allowed to variably disturb the hydrophobic surface in helix 14C. It has been shown that a stretch of 12 amino acid residues (1768–1779) is involved in the PE/PC binding. It appeared that a hydrophobic surface on one side of the β -spectrin helix 14C was formed mostly by residues within this stretch. A decrease in lipid-binding affinity was related to the number of substitutions in the four hydrophobic positions, and the K_d values followed the sequence: wild type < 2 substitutions < 3 substitutions < 4 substitutions. Remarkably, none of the analyzed mutations caused changes in the affinity for ankyrin binding. It has been suggested the minimal ankyrin-dependent PE/PC-binding motif in erythroid β -spectrin is 1771WXXXLXXMW1779.

As mentioned above, the distance between helices A and C of the 14th repeat of β -spectrin was shown to increase upon interacting with membranes or detergents [112]. On top of that, the location of lipid-binding residues proposed by Wolny et al. [104], suggests that the hydrophobic regions of helices 14A and 14C may become available for penetrating PE-rich lipid mono- or bilayers. This would be true only when the helices of the spectrin 14th repeat partially separate from each other. A model based on the obtained data and the available atomic structures of the ankyrin-binding domain suggest that the W1771 residue is slightly shifted outside the hydrophobic core of the triple helical bundle and thus could initiate anchoring of spectrin to the lipid bilayer. Other hydrophobic residues, including L1775, M1778 and W1779, form a compact hydrophobic patch, which supports the proposed model. This model fits to the more general mechanism of conformational flexibility proposed for the N-terminal regions of helix C of spectrin repeats in solution [133] and could explain the accessibility of the lipid-binding site. Moreover, binding of erythroid ankyrin to this domain seems to lock the structure of the triple helical bundle by binding charged residues of helices A and C, which makes this region of spectrin molecule inaccessible for interactions with membranes [104].

Recently published data [104, 134] showed how the full-length ankyrin-binding domain of erythroid spectrin and its truncated or point mutated counterparts influenced the properties of erythrocyte ghosts and HeLa cell membranes. After encapsulation upon resealing of erythrocyte ghosts, proteins comprising the full-length ankyrin-binding domain caused a dramatic change in shape and a decrease in the barrier properties and release of spectrin from the membrane, which was in contrast to the protein truncated from the N-terminus. The changes in permeability and mechanical properties suggested that PE-rich lipid-binding activity is important for maintaining membrane integrity.

The expression of the full-length GFP-tagged ankyrin-binding domain in HeLa cells induced the appearance of aggregates consisting not only of GFP-tagged proteins but also endogenous spectrin together with some transmembrane proteins, which are known to interact with this protein via ankyrin, i.e. Na⁺/K⁺-ATPase, IP3 receptor protein and L1 CAM. By contrast, overexpression of N-terminally truncated or quadruple mutant proteins did not result in such aggregation. Interestingly, the morphology of the actin cytoskeleton and localization of E and N cadherins remained unchanged upon overexpression of each of the mentioned proteins.

These observations may suggest that ankyrin-sensitive lipid-binding domain plays a key role in maintaining the proper distance between the neighboring spectrin tetramers, which allows maintenance of the appropriate distance between the individual members of erythroid ankyrin–AE1 transmembrane protein complexes, therefore preventing their aggregation. It is suggested [104] that inhibition of spectrin–lipid association by overexpression of the wild type ankyrin-binding domain induced the formation of aggregates containing spectrin, ankyrin and transmembrane proteins. The activity of this part of the spectrin molecule affects membrane mechanical and barrier properties.

It is well known that the number of ankyrin molecules is equal to the number of spectrin tetramers in natural membranes, and there are a considerably high amount of unblocked lipid-binding sites. Bearing this in mind, it could be speculated that spectrin–lipid interactions are involved in the maintenance of cellular homeostasis. In conclusion, the suggested model for the ankyrin-binding domain of erythroid β -spectrin presupposes the existence of two possible conformations: the “closed” structure observed when spectrin interacts with ankyrin; and the “open” one, which was detected upon lipid binding.

Another possibility is that the PE-rich lipid-binding activity of the β -spectrin ankyrin-binding domain is linked to the interactions of the membrane skeleton with loosely packed domains of the inner membrane lipid layer. It is known that the latter is enriched in PE, a lipid with a relatively low propensity for lamellar structures due to its small head group. Such intrinsic instability could lead to a locally release during the binding of a protein that alternatively may penetrate the membrane. In other words, the cone-shaped PE molecules make the membranes more prone to penetration via amphipathic lipid-packing sensors [135]. Indeed, the aforementioned lipid-binding site could be classified as this type of peripheral membrane proteins, as they bind with the same or even higher efficiency to neutral than to negatively charged membranes, and they are exceptionally sensitive to lipid-packing defects. This is nicely reflected by the fact that the affinity of spectrin to PE/PC monolayers considerably increases with a decrease in the initial surface pressure, and the protein is able to efficiently penetrate “loose” (approx. 9 mN/m, far from the 30–35 mN/m assigned for natural membranes) PE/PC monolayers [89, 90]. Moreover, both native spectrin [136] and the isolated ankyrin-sensitive PE/PC lipid-binding domain of beta spectrin [103] induced an increase in lipid hydrocarbon chain mobility of either PE/PC vesicles or natural membranes. It could be postulated that spectrin plays an important role in the regulation of the fluidity of the inner lipid leaflet of the plasma membrane and the spatial organization of membrane lipid microdomains.

Concluding remarks

Apparently, over 40 years of studies on spectrin–lipid interactions is not enough to give a clear and consistent picture of the issue. The data presented in this brief review indicate a reasonable progress of research on lipid binding by membrane skeletal proteins, which was achievable not only through the tremendously rapid development of the available toolbox, but also due to recent appreciation of the importance of biological membranes and individual lipid species in cellular processes. Nevertheless, there are still unanswered questions concerning the physiological role of spectrin interactions with lipid bilayers. In particular, further research is required to explore the role of these interactions in various pathologies, such as hemolytic anemia (spectrins and membrane skeleton proteins) and/or muscular dystrophies (dystrophin).

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